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GRAS Notice (GRN) No. 539
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

ORIGINAL SUBMISSION

EAS CONSULTING GROUP

Specializing in FDA Regulatory Matters

GRN 000539

August 8, 2014

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

To Whom It May Concern:

In accordance with proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), I am submitting in triplicate, as the agent to the notifier, Enzymotec Ltd., a GRAS Notification of OmegaPC™, a fish-based lipid extract containing EPA and DHA. Also enclosed is a GRAS panel report setting forth the basis for the GRAS determination.

Please let me know if you have any questions.

Sincerely,

(b) (6)

Edward A. Steele
President and CEO

Enclosures

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GRAS NOTIFICATION

OmegaPC™

I. Claim of GRAS Status

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR 170.36(c)(1)

Enzymotec Ltd. (the notifier) has determined that OmegaPC™, a fish-based lipid extract containing EPA and DHA, is Generally Recognized AS Safe (GRAS), consistent with section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use as a food ingredient. Therefore, the use of OmegaPC is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Edward A. Steele

Agent for:

Enzymotec Ltd.
Sagi 2000 Industrial Park
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ISRAEL
Tel.: 972-74-717177
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B. Name and Address of Notifier:

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As the notifier, Enzymotec Ltd. accepts responsibility for the GRAS determination that has been made for OmegaPC™ as described in the subject notification; consequently, OmegaPC™ meeting the conditions described herein is exempt from premarket approval requirements for food ingredients.

C. Common or Usual Name of the Notified Substance and Identity of GRAS Substance

The common name of the substance of this notification is fish-based lipid extract containing omega-3 fatty acids (EPA + DHA) bound to phospholipids and triglycerides, trade name OmegaPC™. This product is manufactured by Enzymotec Ltd. (Enzymotec). OmegaPC™ is composed primarily of phospholipids and triglycerides , with lesser amounts of diglycerides, with the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) predominating at about 10 percent and 12 percent, respectively, yielding a combined EPA and DHA content of approximately 22 percent by weight. This level is comparable to the total of EPA plus DHA (22%) given by the FDA for menhaden oil. The fish source employed as the starting material in the production of OmegaPC™ is fish meal obtained from multiple edible fish species including anchovies (*Engraulis sp., e.g. Engraulis ringens*), sand eel (*Hyperoplus sp., Gymnamodytes sp. or Ammodytes sp., e.g. Ammodytes tobianus*), sprat (*Sprattus sprattus*), herring (*Clupea sp., e.g. Clupea harengus*), boar fish (*Capros aper*), Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*micromesistius poutassou*) and/or Jack Mackerel (*trachurus murphyi*) , Sardine *(Sardinops sagax)*and other suitable species.

The fatty acid profile for OmegaPC™ is presented in Table I below.

Table I. Fatty Acid Profile for Omega PC™	
Fatty acid	**Typical value (as % of total fatty acids)**
C14 (Myristic)	6
C16 (Palmitic)	19
C16:1n7 (Palmitoleic)	6
C18:0 (Stearic)	4
C18:1n9 (Oleic)	8
C18:1 (Octadecenoic)	3
C18:4n3 (Octadecatetraenoic)	2
C20:1n9 (Eicosenoic)	2
C20:5n3 (Eicosapentaenoic) (EPA)	**16**
C22:5n3 (Docosapentaenoic)	2
C22:6n3 (Docosahexaenoic) (DHA)	**18**
Others	14
Sum	100

The fatty acid profile of OmegaPC™, summarized above, is generally consistent with other naturally occurring marine oils. The compositional analysis of the product supports the presumption that OmegaPC™ is metabolized by the body similarly to other naturally occurring fish oils and poses no novel safety concerns.

OmegaPC™ derived from edible fish meal employs a proprietary extraction process that is not substantially different from extraction processes employed in this segment of the fish industry. The process employs good manufacturing practice procedures. This process can be compared with the other extraction processes used to obtain the fish oils described in the GRAS Notices that FDA has received for refined fish oils. The process employed is consistent with the guidelines enunciated in the FDA guidance entitled "Draft Guidance for Industry: Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives" available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm300661.htm.

D. Conditions of Use and Consumer Exposure

Enzymotec intends to market OmegaPC™ as a nutrient (21 CFR 170.3(o)(20) to increase the dietary intake of the two omega-3 fatty acids, EPA and DHA. OmegaPC™ will be added to those food categories at concentrations providing the same combined EPA and DHA content as are permitted for menhaden oil under 21 CFR 184.1472. OmegaPC™ will be used as the sole source of EPA and DHA in any given food category of this regulation and will not be combined or augmented with any other EPA/DHA-rich oil in making a food product. Based on EPA+DHA content of 22 percent in menhaden oil and 22 percent in OmegaPC™, the corresponding maximum proposed use levels are estimated to be equivalent to the uses as specified for that of menhaden oil. These proposed uses are presented in Table II. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions (GRN Nos. 105, 109, 138, 146, 193, and 200) as well as GRAS Notices recognizing the use of EPA/DHA from other sources (GRNs 319, 335, 137, 226, 371, 279 and 311). Because the combined EPA and DHA content of foods to which OmegaPC™ will be added is identical to that permitted for menhaden oil, OmegaPC™ will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of OmegaPC™.

The estimated mean intake of EPA and DHA combined from all dietary sources in the general population, excluding infants under the age of one year, has been addressed in earlier FDA rulemaking (menhaden oil final rule; 62 FR 30751; June 5, 1997) where FDA estimated that the mean exposure to EPA and DHA from the use of menhaden oil in all food categories would be 2.8 grams/person/day. It was also addressed as well in the subsequent GRAS Notices referenced above. The total cumulative estimated mean intake of EPA and DHA combined from the proposed maximum use levels of OmegaPC™ listed in the foods in Table II is also estimated to be 2.8 grams per person per day. This estimate reflects 100 percent market penetration of the proposed uses of OmegaPC™ that are listed in Table II. Because 100 percent market penetration of this product is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower. Further, this cumulative intake of EPA and DHA combined is still less than the safe limit for EPA and DHA combined of 3 grams per person per day established by the agency.

E. Basis for the GRAS Determination

The GRAS determination for OmegaPC™ under the proposed maximum use levels listed in Table II is based on scientific procedures as described under Title 21 of the Code of Federal Regulations (21 CFR 170.30(b). These scientific procedures have been used to demonstrate that the estimated intake of OmegaPC™ from the intended uses specified in Table II is safe, and also GRAS under the Food, Drug, and Cosmetic Act (FDCA). FDA has already established that a combined intake of EPA and DHA of less than 3 grams per person per day is a safe level of intake. In addition, this cumulative intake of EPA and DHA combined was also determined to be GRAS and the safety of this level of intake is generally recognized by experts qualified by both training and experience to evaluate the safety of substances directly or indirectly added to food, and is also based on generally available and accepted information.

Table II. Proposed Maximum Use Levels in Food as Served of OmegaPC™ Compared with Current Menhaden Oil Uses Permitted under 21 CFR 184.1472 and Final Rule Published in 70 FR 14531; March 23, 2005

Category of food (and Maximum Level of Use of 21CFR 170.3(n) paragraph)	Maximum Level of Use of Menhaden Oil under 21 CFR 184.1472 and 70 FR 14531; March 23, 2005	Proposed Maximum Level of Use of OmegaPC™
Baked goods, baking mixes, 21 CFR 170.3(n)(1)	5.0%	5.0%
Cereals, 21 CFR 170.3(n)(4)	4.0%	4.0%
Cheese products, 170.3(n)(5)	5.0%	5.0%
Chewing gum, 170.3(n)(6)	3.0%	3.0%
Condiments, 170.3(n)(8)	5.0%	5.0%
Confections, frostings, 170.3(n)(9)	5.0%	5.0%
Dairy product analogs, 170.3(n)(10)	5.0%	5.0%
Egg products, 170.3(n)(11)	5.0%	5.0%
Fats, oils, 170.3(n)(12)	12.0%	12.0%
Fish products, 170.3(n)(13)	5.0%	5.0%
Frozen dairy desserts, 170.3(n)(20)	5.0%	5.0%
Gelatins, puddings, 170.3(n)(22)	1.0%	1.0%
Gravies, sauces, 170.3(n)(24)	5.0%	5.0%
Hard candy, 170.3(n)(25)	10.0%	10.0%
Jams, jellies, 170.3(n)(28)	7.0%	7.0%
Meat products, 170.3(n)(29)	5.0%	5.0%
Milk products, 170.3(n)(31)	5.0%	5.0%
Nonalcoholic beverages, 170.3(n)(3)	0.5%	0.5%
Nut products, 170.3(n)(32)	5.0%	5.0%
Pastas, 170.3(n)(23)	2.0%	2.0%
Plant protein products, 170.3(n)(33)	5.0%	5.0%
Poultry products, 170.3(n)(34)	3.0%	3.0%
Processed fruit juices,	1.0%	1.0%

170.3(n)(36)		
Snack foods, 170.3(n)(37)	5.0%	5.0%
Soft candy, 170.3(n)(38)	4.0%	4.0%
Soup mixes, 170.3(n)(40)	3.0%	3.0%
Sugar substitutes, 170.3(n)(42)	10.0%	10.0%
Sweet sauces, toppings, syrups, 170.3(n)(43)	5.0%	5.0%
White granulated sugar, 170.3(n)(41)	4.0%	4.0%

The safety of consumption of OmegaPC™ for use as an ingredient in food is based on the similarity of this product's composition to other currently GRAS marketed fish-oil derived products as well as other GRAS-derived products that contain EPA/DHA and the established safety of ingestion of its two major fatty acid constituents, EPA and DHA. The safety of consumption of OmegaPC™ was determined by evaluating the source of the product, the production process, the nature and quantity of impurities, product specifications, and the identity and positional distributions of EPA and DHA within the lipids that comprise the final product. Appropriate specifications have been established to ensure that the final product is food grade. Compositional analysis of the product supports the presumption that there is no toxicological concern from the ingestion of any product impurities.

In affirming the GRAS status of menhaden oil under 21 CFR 184.1472, the FDA established that a daily intake of EPA and DHA combined not exceeding 3 grams per person per day is safe. The scientific basis to support the establishment of this safe level of intake has been published by the FDA. In 1997, FDA affirmed, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use to ensure that the total daily intake of EPA and DHA would not exceed 3.0 grams per person per day (g/p/d) (62 FR 30751; June 5, 1997; 21 CFR 184.1472). EPA and DHA are the major omega-3 fatty acids in fish oil and together comprise about 20 percent by weight of menhaden oil. FDA established maximum use levels of menhaden oil in certain foods because of concerns over possible adverse effects of fish oil consumption on bleeding time, glycemic control, and LDL cholesterol (62 FR 3075 1 at 30757; June 5, 1997). In 2002, FDA published a proposed rule to reallocate the uses of menhaden oil in conventional food, while maintaining the total daily intake of EPA and DHA from menhaden oil at a level not exceeding 3.0 g/p/d (67 FR 8744; February 26, 2002). FDA placed specific limitations, including the category of foods, the functional use of the ingredient, and the level of use, to ensure that the consumption of EPA and DHA from conventional food sources would not exceed 3.0 g/p/d. FDA then published a tentative final rule (69 FR 23 13; January 15,2004) to additionally require that menhaden oil not be used as an ingredient in foods in combination with other added oil that is a significant source of EPA and DHA to ensure that total intake from conventional food sources do not exceed 3 .0 g/p/d. The mean exposure to EPA and DHA from menhaden oil in all conventional food categories

is estimated to be 2.7 g/p/d (67 FR 8744 at 8746; February 26, 2002). Not all foods in the marketplace within those permitted food categories would contain menhaden oil or other sources of EPA and DHA omega-3 fatty acids that substitute for other edible fat or oil. Also, because not all foods that a consumer eats every day would contain menhaden or other EPA and DHA oil used as a substitute oil, the actual total daily intakes of EPA and DHA from menhaden or other EPA and DHA oil for an average person should be significantly below 3.0 g/p/d (67 FR 8744 at 8746; February 26, 2002). [NOTE: the finalized allocation has since been published in a final rule on March 23, 2005, 70 FR 14530 and is codified at 21 CFR 184.1472)].

This is a conservative estimate with a substantial margin for safety, and the agency believes, consistent with its prior decision on the use of a qualified health claim for DHA and EPA omega-3 fatty acids (October 31, 2000 letter) for dietary supplements, that the addition of menhaden oil to food products has not come close to this conservative mean estimate exposure. FDA further believes that the GRAS uses for which it received a GRAS notification for other sources of EPA and DHA omega-3 fatty acids also provide conservative estimates of exposure and that the addition of these EPA and DHA sources to food products do not come close to the conservative mean estimates.

On September 8, 2004, the requested use of the qualified health claim for omega-3 fatty acids was extended to conventional foods. The agency believes that there is likely to be some increase in consumption of EPA and DHA omega-3 fatty acids based on conventional foods that bear the qualified health claim; however, the amounts of EPA and DHA that can be used and the foods in which such food ingredients can be safely used are limited. Also, manufacturers that have submitted GRAS notifications for other sources, to which the agency has not objected, have established conditions of use similar to those in the menhaden oil GRAS rule.

Based on the data and information that FDA considered, which includes data and information that FDA relied upon in reaching its conclusions about the safety of EPA and DHA omega-3 fatty acids in its GRAS affirmation of menhaden oil, and the data and information in the 1991 proposed (56 FR 60663; November 27,1991) and 1993 final rules (58 FR 2683; January 6, 1993), and its current scientific literature review for other possible safety concerns, FDA concluded that the use of EPA and DHA omega-3 fatty acids when used as a GRAS ingredient, consistent with FDA's GRAS rule for menhaden oil and GRAS notifications to which FDA did not object, and the use as a dietary supplement is safe and lawful under 21 CFR 101.14 provided that daily intakes of EPA and DHA omega-3 fatty acids from conventional food and dietary supplement sources do not exceed 3.0 g/p/d.

The OmegaPC™ product that is the subject of this safety assessment is comprised primarily of phospholipids and triglycerides of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). It is known by the commercial name of OmegaPC™. The intended applications of this omega-3 product will be for those uses in foods for which menhaden oil is permitted under 21 CFR 184.1472 and as noted in the Final Rule (70 FR 14530 – 14532; March 23,

2005). The maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. These proposed uses are presented in Table II above. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above. Because the combined EPA and DHA content of foods to which OmegaPC™ will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, OmegaPC™ will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of OmegaPC™. Therefore, OmegaPC™ can be considered safe under its intended conditions of use.

Enzymotec has also conducted a review of the scientific literature published since the final rule appeared in the Federal Register on March 23, 2005 (70 FR 14530) affirming the GRAS status of menhaden oil and confirmed the conclusion reached by the FDA that safety of ingestion of up to 3 grams per person per day of EPA and DHA combined is consistent with current information regarding the safety of consumption of these two omega-3 fatty acids. No new safety issues apart from FDA's original 3 concerns on bleeding time, glycemic control effects in diabetes and elevated LDL levels in populations with hypertriglyceridemia or hypercholesterolemia were identified. A synopsis of the information uncovered in this literature review is presented below. While the recent research reports focused primarily on the clinical usefulness and efficacy of EPA and DHA, the finding continue to support the fact that the current uses of EPA and DHA in products like OmegaPC™ is safe.

From the foregoing analysis and rulemaking decisions of FDA on the GRAS affirmation of menhaden oil and of EPA and DHA, as well as on the submitted GRAS Notices where the agency had no objection to the conclusions of EPA and DHA being GRAS, Enzymotec's OmegaPC™ product should also be GRAS for the proposed uses specified in the regulations under the conditions described and at the maximum use levels described in Table II.

Based on a critical evaluation of the publicly available data and information summarized in the attached GRAS determination, the Expert Panel members, have individually and collectively concluded that OmegaPC™ meeting the specifications cited above, is GRAS when used as an ingredient in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation when used at a levels equivalent to that of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined. It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that OmegaPC™, when used as described, is GRAS based on scientific procedures.

F. Availability of Information

The detailed data and information that serve as a basis for this GRAS determination will be provided to the FDA upon request, or are available for the Food and Drug Administration's review and copying during reasonable business hours at the offices of:

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1700 Diagonal, suite 750
Alexandria, VA 22314
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II. Detailed Information about the Identity of the Substance

OmegaPC™, a fish-based lipid extract containing EPA and DHA, is a standardized product obtained from fish meal.

A. Identity:

This product has no single chemical name as it is a mixture of phospholipids and triglycerides of various long chain fatty acids, with small amounts of mono and diglycerides . The primary components are triglycerides and phospholipids that include EPA and DHA. The CAS registry number for fish oils is 8016-13-5. The amount of EPA and DHA in OmegaPC™ would be not less than 18% w/w.

B. Trade Name:

The subject of this notification will be marketed as OmegaPC™.

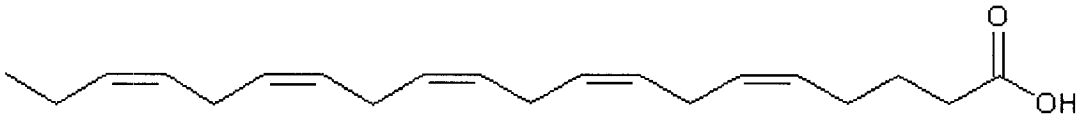
C. Chemical Abstracts Registry Number:

Because the lipid extract OmegaPC™ that is the subject of this GRAS notification is a mixture of phospholipids and triglycerides of various long chain fatty acids with small amounts of mono and diglyceridess, no Chemical Abstracts Service (CAS) Registry Number exists for this substance. The CAS Registry Numbers for EPA and DHA, the primary components of this product, are 104 17-94-4 and 25 167-62-8, respectively.

D. Chemical Formula:

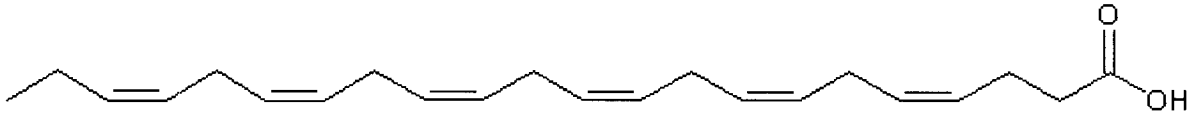
E. Structure:

Figure 1. Structural Formulas for EPA and DHA



Eicosapentaenoic Acid (EPA) (20:5 n-3)

Figure by RMB



Docosahexaenoic Acid (DHA) (22:6 n-3)

Figure by RMB

F. Molecular Weight:

Since OmegaPC™ is a mixture comprised of different substances, no molecular weight for the product is established.

G. Physical Characteristics:

OmegaPC™ is a viscous liquid with a dark brown color and characteristic fishy odor.

H. Typical Composition and Specifications

Fish meal, the source material for the production of OmegaPC™, is a biomass composed of lipids, sugars and proteins. By using a solvent extraction process, the proteins and free sugars are removed so that only lipids are left. Fish meal is generally produced from fresh or frozen fish through cooking, followed by separation into a solid and a liquid phase, usually by pressing. The solid phase is further dried in an industrial dryer to a moisture content of 5-15% to produce the final fish meal. Fish meal used as a source material for the production of OmegaPC™ is derived from multiple edible marine fish species and is obtained from commercial fish production plants. These fish species include anchovies (*Engraulis sp., e.g. Engraulis ringens*), sand eel (*Hyperoplus sp., Gymnamodytes sp. or Ammodytes sp., e.g. Ammodytes tobianus*), sprat

(*Sprattus sprattus*), herring (*Clupea sp., e.g. Clupea harengus*), boar fish (*Capros aper*), Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*micromesistius poutassou*) and/or Jack Mackerel (*trachurus murphyi*) , Sardine *(Sardinops sagax)* Codfish, Haddock, Saithe, Menhaden, Salmon and other suitable species.

Typical food grade specifications for OmegaPC™ are presented in Table III. Analytical data from five non-consecutive lots are presented in Appendix I. General product specifications of OmegaPC™ is presented in Table IV.

Table III. Typical Composition of Omega PC

Parameter	Typical values	Assay method
Phospholipids	39% w/w	^{31}pNMR
Neutral lipids		
Triglycerides	39 % w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Diglycerides	8 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Monoglycerides	1 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Free fatty acids	7 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Other neutral lipids	1 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Total neutral lipids	55% w/w	
DHA	12 %w/w	USP Monograph "Fish Oil Containing Omega-3 Acids"
EPA	10 %w/w	USP Monograph "Fish Oil Containing Omega-3 Acids"
Cholesterol	24 mg/g	EP 2.4.32
Heavy metals		
Lead	<0.05 ppm	Ph. Eu. Method 2.4.27
Arsenic (total)	16 ppm	Ph. Eu. Method 2.4.27
Arsenic (inorganic)	0.01 ppm	EPA Method 1632 (mod.)
Cadmium	0.01 ppm	Ph. Eu. Method 2.4.27
Mercury	<0.005 ppm	Ph. Eu. Method 2.4.27

Table IV. Product specifications for OmegaPC™

Parameter	Specifications	Assay method
Consistency	Viscous liquid	Visual
Color	Dark brown	Visual
Peroxide value	< 5 meq/Kg	USP 401
Moisture	< 4 % w/w	USP 921
Phospholipids	> 35% w/w	^{31}pNMR
DHA+EPA	> 18 % w/w	USP Monograph "Fish Oil Containing Omega-3 Acids"
Ethanol residues	< 5000 ppm	GC-FID
Hexanes residues	< 5 ppm	GC-FID
Microbiological assays		
Total plate count	< 1000 cfu/g	Israeli Standard SI 885 Part 3 (1999)
Yeast and Mold	< 100 cfu/g	Israeli Standard SI 885 Part 3 (1999)
Molds	< 100 cfu/g	Israeli Standard SI 885 Part 3 (1999)
Coliforms	Negative (cfu/g)	USP 61 (2000)
Staphylococcus aureus	Negative (cfu/g)	USP 61 (2000)
Salmonella	Negative (cfu/20g)	Israeli Standard SI 885 Part 3 (1999)
Shelf life	24 months	
cfu=colony forming units		

Although specifications were not established for polyaromatic hydrocarbons (PAH), analyses were conducted for them as we aware of concerns associated with the presence of these compounds. This data is shown in Appendix I. None were present at levels of toxicological concern. It should be noted that PAH contamination is generally not considered to be an issue for standard fish oils although it might be a problem in smoked fish products.

I. Manufacturing Process

OmegaPC™ is produced through solvent extraction of fish meal. Fish meal, a biomass composed of lipids, sugars and proteins, is generally produced from fresh or frozen fish by cooking followed by separation into a solid and a liquid phase, usually by pressing. The solid phase is further dried in an industrial dryer to a moisture content of 5-15% to produce the final fish meal. The fish meal is inspected for acceptability prior to being extracted.

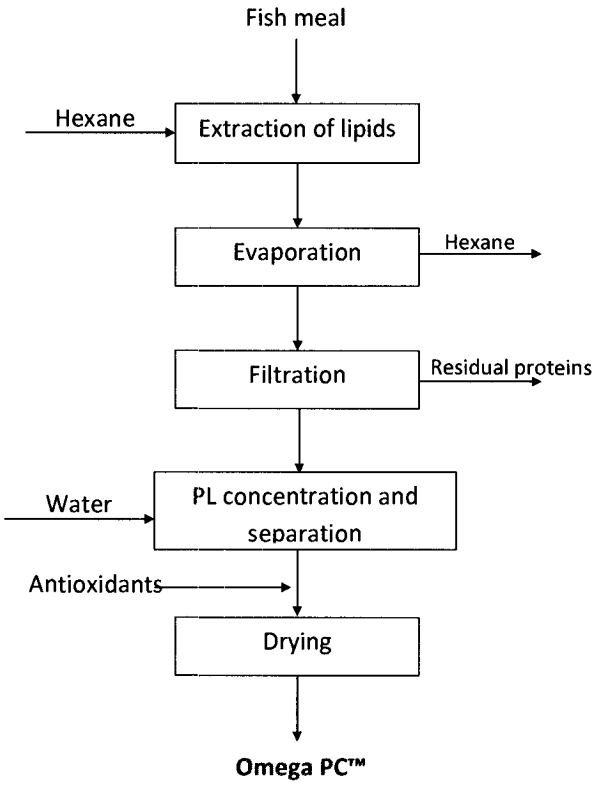
Lipids from the fish meal are extracted continuously using hexane meeting the specifications in the Food Chemicals Codex, 5th Ed. Following the solvent extraction process, the liquid organic phase, which contains the solvent and the extracted lipids, undergoes vacuum evaporation in order to remove the solvent. The crude oil, which contains phospholipids and triglycerides , is then filtered in order to remove residual proteins and other impurities. Following the filtration stage, the phospholipids are concentrated by mixing the crude oil with water and subjecting it to

centrifugation to provide a phospholipid-rich phase (the crude product) and a phospholipid-poor phase (fish oil). The phospholipid-rich phase is dried from residual water by vacuum evaporation and may further be mixed with fish oil for standardization . Food grade antioxidants are then added to the product in accordance with good manufacturing practice. The process is conducted in a nitrogen-rich environment in order to maintain the stability of the product throughout the production process.

Analyses of four non-consecutive batches (Appendix I) demonstrate that the manufacturing process results in product that consistently meets product specifications. An overview of the manufacturing process for OmegaPC™ is shown below.

J. Manufacturing Process Diagram

Figure 1: Process Flow Diagram



K. Intended Technical Effects

Enzymotec intends to market its OmegaPC™ product for addition to several categories of foods as a nutrient supplement (21 CFR 170.3(o)(20)) to increase the dietary intake of the omega-3 fatty acids EPA and DHA. The food categories proposed for addition and the proposed addition levels are listed in Table II above. These are the same food categories as are specified in the GRAS regulation for menhaden oil (21 CFR 184.1472(a)(3)), and OmegaPC™ thus serves as an alternative to menhaden oil and other fish oils as a source of EPA and DHA. OmegaPC™ is proposed for addition at the same use levels proposed for menhaden oil (also shown in Table II), reflecting the average 22% EPA+DHA composition of OmegaPC™ compared with 22% EPA and DHA in menhaden oil. Thus, the addition rates of EPA+DHA are the same for OmegaPC™ as for menhaden oil.

III. Summary of the Basis for the Notifier's Determination that OmegaPC™ is GRAS

Enzymotec's GRAS determination for the proposed uses of its OmegaPC™ product listed in Table II is based on scientific procedures as described under 21 CFR 170.30(b). Enzymotec's OmegaPC™ has been shown to be substantially equivalent to other edible fish oils (see Table I and Appendix I), including fish oils that are already GRAS for addition to foods. The fact that the use of OmegaPC™ is substitutional means that there is no increase in exposure to omega-3 fatty acids resulting from the addition of OmegaPC™ to food. Enzymotec has conducted an updated literature search to establish that no new safety concerns exist for the use of OmegaPC™. Based on the totality of the evidence, Enzymotec has concluded that the intended use of OmegaPC™ is GRAS and that other scientists, competently trained, would concur.

The FDA has previously reviewed the safety of consumption of fish oil containing the two omega-3 fatty acids EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS under specified conditions of use (FDA 1997). According to the FDA, the primary safety concerns associated with excessive intakes of EPA and DHA include increased bleeding times, reduced glycemic control among diabetics, and increased levels of low-density lipoprotein (LDL) cholesterol among diabetics and hyperglycemics. Enzymotec has expanded upon FDA's evaluation and reviewed the more recent literature to determine if more current information pertaining to these safety concerns would contradict what was concluded and recommended in the 1997 FDA opinion regarding EPA and DHA intake from fish oil. This review has focused on the safety of fish oil and of intake of EPA+DHA combined rather than on the distinct metabolic effects of EPA and DHA in isolation.

FDA has also received and reviewed several GRAS Notices regarding fish oil products containing EPA and DHA as well as GRAS Notices related to EPA and DHA derived from algae and krill. In all of these GRAS Notices, after review, FDA issued a letter informing the notifier that they had "No questions" concerning their conclusion that their products containing EPA and DHA were GRAS. The fact that FDA has not expressed any concerns with these products coming from different sources and different production processes

FDA is comfortable with the safety of these products. A list of these GRAS
:d below in Table V. As the information in these GRAS Notices directly
. ...e safety of OmegaPC™, these notices are incorporated by reference into this
...AS Notice.

Table Va. GRAS Notices received by FDA related to EPA and DHA

GRN No.	Submitted by	Subject of notice	Daily intake of DHA+EPA
105	Unilever	Fish oil	Use consistent with 21 CFR 184.1472
109	Clover Corporation Limited	Fish oil	
138	Ocean Nutrition Canada	Fish oil	
146	Jedwards International	Fish oil	
193	Peluva Biotech	Fish oil	
200	Twin Rivers Technologies	Tailored triglycerides enriched in omega-3 fatty acids from fish oil	
319	Lonza Ltd.	Micro-algal oil *Ulkenia*	7.9 g/p/d
355	DuPont Applied Biosciences	EPA-rich triglyceride oil from *Yarrowia lipolytica*	3.0 g/p/d
137	Martek	Algal oil (*Schizochytrium)*	1.5 g/p /day (mean intake)
226	Enzymotec	Krill lecithin	Up to 3g/p/d
242	Neptune	Krill oil	2.2 g/p/d
371	Aker Biomarine	Krill oil	1.95 g/p/d
279	Enzymotec	Fish-based PS	35 mg/p/d (90th percentile)
311	Enzymotec	Krill-based PS	33 mg/p/d (90th percentile)

Table Vb. GRAS Notices received by FDA related to lecithin/PC

GRN No.	Submitted by	Subject of notice	Daily intake of PL
226	Enzymotec	Krill-derived lecithin	Not calculated. Not referred to in the FDA's letter.
242	Neptune	Krill oil	Not calculated, but based on daily consumption of 8.3 g NKO/d (Table 15) and an average level of 45.3 g PL/100 g NKO (Table 2), the

			upper consumption of PL is calculated as 3.76 g/d
371	Aker Biomarine	Krill oil	Not indicated, but based on EDI of 8.28g Krill oil/d (90^{th} percentile) and a level of 43% PL in Krill oil, the upper consumption level of PL is calculated as 3.56g/d.

IV. Basis for a Conclusion that OmegaPC™ is GRAS for its Intended Use

The publicly available data demonstrating the safety of the proposed uses of OmegaPC™ was reviewed by an independent GRAS panel consisting of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients. Based on a critical evaluation of the pertinent data and information summarized herein, the Expert Panel members have individually and collectively determined, by scientific procedures, that the use of OmegaPC™ in food categories identified in Table II above at levels permitted in the regulation for menhaden oil (21 CFR 184.1472) is GRAS. It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion (see the attached Expert Panel statement.)

EXPERT PANEL STATEMENT
DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF OmegaPC™ AS A Nutrient supplement

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August 2014

EXPERT PANEL STATEMENT DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF OmegaPC™

AS A Nutrient Supplement

TABLE OF CONTENTS

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF OmegaPC™ AS A NUTRIENT SUPPLEMENT

1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by EAS Consulting Group, LLC (EAS) at the request of Enzymotec Ltd. (Enzymotec) to determine the Generally Recognized As Safe (GRAS) status of OmegaPC™ as a nutrient supplement [21 CFR 170.3(o)(20)] in selected food products as identified in 21 CFR 184.1472. A comprehensive search of the scientific literature for safety and toxicity information on OmegaPC™ and similar products was conducted through January 2014 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Enzymotec and other information deemed appropriate or necessary. Enzymotec assures that all unpublished information in its possession and relevant to the subject of this determination has been provided to EAS and has been summarized accurately in this GRAS monograph. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

1.1. Background

Oils containing EPA and DHA are known to occur naturally in many marine food sources, including fish, shellfish, krill, and algae. Many of these oils, derived from these sources, are used in foods and dietary supplements to supply EPA and DHA to the diet. An excellent source for obtaining EPA and DHA-rich oils is fish biomass obtained from edible fish. An analysis of lipid classes, fatty acids, and sterols in samples of fish and seafood from Gilbert Bay, South Labrador has been conducted (Copeman and Parrish, 2004). These results are summarized in Table 1. The Phospholipid (PL) fraction in fish and seafood can account for up to 75% of total lipids, with the flesh of fish generally having a greater composition than fish livers. These data suggest that a 100 g serving of cooked blue mussels, which contains 4.48 g of fat (USDA[1]) would provide approximately 1.69 g PL, and 1.6g triglycerides (TG). Similarly, a 100 g serving of cooked Atlantic herring, which contains 0.86 g of fat (USDA[2]) would provide approximately 0.47 g PL and 0.1g TG. Considering an upper daily intake of 2.8 g DHA+EPA and a level of 22% w/w DHA+EPA in OmegaPC™, the daily intake of OmegaPC™ would be 13g. Phospholipids and triglycerides account for 40 and 36% of OmegaPC™, respectively. Therefore, the upper daily

[1] http://ndb.nal.usda.gov/ndb/foods/show/4624

[2] http://ndb.nal.usda.gov/ndb/foods/show/4503?fg=&man=&lfacet=&format=&count=&max=25&offset=&sort=&qlookup=herring

intake of phospholipids and triglycerides resulting from the consumption of OmegaPC™ would be about 5g phospholipids and 4.7g triglycerides.

Table 1	**Phospholipid and triglycerides composition of various species of fish and seafood from Gilbert Bay, South Labrador**			
Species	Phospholipids (% of total lipids)		Triglycerides (% of total lipids)	
Fish	Flesh	Liver	Flesh	Liver
Northern cod, G. morhua	54.9 ± 6.5	12.3 ± 7.0	11.4 ± 3.5	66.9 ± 13.8
Golden cod, G. morhua	55.5 ± 3.3	13.3 ± 4.4	12.2 ± 0.2	55.0 ± 9.1
Rock cod, G. ogac	43.9 ± 5.4	11.6 ± 2.3	15.9 ± 2.4	66.2 ± 4.4
Herring, C. harengus	6.4 ± 2.1	35.8 ± 7.8	86.3 ± 1.9	19.6 ± 10.5
Seafood	Whole animal		Whole animal	
Surf clam, S. solidissima	63.3		0.0	
Greenland cockle, S. groenlandicus	49.9 ± 7.6		14.3 ± 6.5	
Blue mussel, M. edulis	37.8 ± 3.1		34.7 ± 8.5	
Icelandic scallop, C. islandica	74.6 ± 3.7		0.5 ± 0.8	

Adopted from Copeman and Parrish, 2004.

1.2. Description

The common name of the substance that is the subject of this Generally Recognized As Safe (GRAS) notification is fish-based lipid extract, trade named OmegaPC™, which is a wild fish lipid extract containing omega 3 fatty acids bound to phospholipids and triglycerides . Fish meal, the source material in the production of OmegaPC™, is extracted from multiple edible marine fish, including anchovies (*Engraulis sp., e.g. Engraulis ringens*), sand eel (*Hyperoplus sp., Gymnamodytes sp. or Ammodytes sp., e.g. Ammodytes tobianus*), sprat (*Sprattus sprattus*), herring (*Clupea sp., e.g. Clupea harengus*), boar fish (*Capros aper*), Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*micromesistius poutassou*) and/or Jack Mackerel (*trachurus murphyi*) , Sardine *(Sardinops sagax)*, Codfish, Haddock, Saithe, menhaden, salmon and other suitable species. . Approximately 22% (on average) of OmegaPC™ is composed of the two omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the form of phospholipids and triacylglycerides . The total content of EPA+DHA would be not less than 18% w/w of the product. OmegaPC™ is substantially similar to other edible fish oils, as shown by the comparison of fatty acid profiles shown in Table I. It is also substantially similar to other fish oils that are already regarded as GRAS (Table 2) for addition to foods, including menhaden oil (21 CFR 184.1472), small planktivorous pelagic fish body oil (SPPFBO, GRAS Notice GRN 102;

FDA 2002) and krill (GRNs 226, 242) (Table 2). This latter fish oil is derived primarily from sardine and anchovy, the same fish that is part of the fish types used to produce OmegaPC™.

Table 2a. GRAS Notices received by FDA related to EPA and DHA

GRN No.	Submitted by	Subject of notice	Daily intake of DHA+EPA
105	Unilever	Fish oil	Use consistent with 21 CFR 184.1472
109	Clover Corporation Limited	Fish oil	
138	Ocean Nutrition Canada	Fish oil	
146	Jedwards International	Fish oil	
193	Peluva Biotech	Fish oil	
200	Twin Rivers Technologies	Tailored triglycerides enriched in omega-3 fatty acids from fish oil	
319	Lonza Ltd.	Micro-algal oil *Ulkenia*	7.9 g/p/d
355	DuPont Applied Biosciences	EPA-rich triglyceride oil from *Yarrowia lipolytica*	3.0 g/p/d
137	Martek	Algal oil (*Schizochytrium)*	1.5 g/p /day (mean intake)
226	Enzymotec	Krill lecithin	Up to 3g/p/d
242	Neptune	Krill oil	2.2 g/p/d
371	Aker Biomarine	Krill oil	1.95 g/p/d
279	Enzymotec	Fish-based PS	34.38 mg/p/d (90^{th} percentile)
311	Enzymotec	Krill-based PS	33 mg/p/d (90^{th} percentile)

Table 2b. GRAS Notices received by FDA related to lecithin/PC

GRN No.	Submitted by	Subject of notice	Daily intake of PL
226	Enzymotec	Krill-derived lecithin	Not calculated. Not referred to in the FDA's letter.
242	Neptune	Krill oil	Not calculated, but based on daily consumption of 8.3g NKO/d (Table 15) and an average level of 45.3g PL/100g NKO (Table 2), the upper consumption of PL is calculated as

			3.76g/d
371	Aker Biomarine	Krill oil	Not indicated, but based on EDI of 8.28g Krill oil/d (90^{th} percentile) and a level of 43% PL in Krill oil, the upper consumption level of PL is calculated as 3.56g/d.

Enzymotec intends to market its OmegaPC™ product for addition to several categories of foods as a nutrient supplement (21 CFR 170.3(o)(20)) to increase the dietary intake of the omega-3 fatty acids EPA and DHA. The food categories proposed for addition and the proposed addition levels are listed in Table 3. These are the same food categories as are specified in the GRAS regulation for menhaden oil (21 CFR 184.1472(a)(3)), and OmegaPC™ thus serves as an alternative to menhaden oil as a source of EPA and DHA. OmegaPC™ **is** proposed for addition at the same use levels proposed for menhaden oil (also shown in Table 3), reflecting the average 22% EPA+DHA composition of OmegaPC™ compared with 22% EPA and DHA in menhaden oil. Thus, the addition rates of EPA+DHA are the same for OmegaPC™ as for menhaden oil.

It is intended that OmegaPC™ will be used as the sole added source of EPA and DHA in any given food category and is not to be combined or augmented with any other source of EPA or DHA in making a food product. Therefore, the overall consumer exposure to EPA and DHA will not change as OmegaPC™ is expected to be a substitute for menhaden oil and other EPA/DHA products.

On February 26, 2002, FDA issued a proposed rule (67 FR 8744) that would amend 21 CFR 184.1472(a)(3) by reallocating the uses of menhaden oil in a different set of food categories, each with a specified maximum level of use. Enzymotec intends that any changes to the permitted uses of menhaden oil specified in 21 CFR 184.1472(a)(3) would also apply to OmegaPC™. In other words, the levels of use of OmegaPC™ would be the same as whatever maximum levels of use are specified in 21 CFR 184.1472(a)(3); in both cases, the permitted categories of foods would be the same. These potential future levels of use are shown in Table 3. **As** with the use of menhaden oil, the maximum level of use of OmegaPC™ is designed to assure that the combined daily intake of EPA and DHA will not exceed 3 g/person/day.

Table 3. Proposed Maximum Use Levels in Food as Served of OmegaPC™ Compared with Current Menhaden Oil Uses Permitted under 21 CFR 184.1472 and Final Rule Published in 70 FR 14531; March 23, 2005

Category of food (and Maximum Level of Use of 21CFR 170.3(n) paragraph)	Maximum Level of Use of Menhaden Oil under 21 CFR 184.1472 and 70 FR 14531; March 23, 2005	Proposed Maximum Level of Use of OmegaPC
Baked goods, baking mixes, 21 CFR 170.3(n)(1)	5.0%	5.0%
Cereals, 21 CFR 170.3(n)(4)	4.0%	4.0%
Cheese products, 170.3(n)(5)	5.0%	5.0%
Chewing gum, 170.3(n)(6)	3.0%	3.0%
Condiments, 170.3(n)(8)	5.0%	5.0%
Confections, frostings, 170.3(n)(9)	5.0%	5.0%
Dairy product analogs, 170.3(n)(10)	5.0%	5.0%
Egg products, 170.3(n)(11)	5.0%	5.0%
Fats, oils, 170.3(n)(12)	12.0%	12.0%
Fish products, 170.3(n)(13)	5.0%	5.0%
Frozen dairy desserts, 170.3(n)(20)	5.0%	5.0%
Gelatins, puddings, 170.3(n)(22)	1.0%	1.0%
Gravies, sauces, 170.3(n)(24)	5.0%	5.0%
Hard candy, 170.3(n)(25)	10.0%	10.0%
Jams, jellies, 170.3(n)(28)	7.0%	7.0%
Meat products, 170.3(n)(29)	5.0%	5.0%
Milk products, 170.3(n)(31)	5.0%	5.0%
Nonalcoholic beverages, 170.3(n)(3)	0.5%	0.5%
Nut products, 170.3(n)(32)	5.0%	5.0%
Pastas, 170.3(n)(23)	2.0%	2.0%
Plant protein products, 170.3(n)(33)	5.0%	5.0%
Poultry products, 170.3(n)(34)	3.0%	3.0%
Processed fruit juices, 170.3(n)(36)	1.0%	1.0%
Snack foods, 170.3(n)(37)	5.0%	5.0%
Soft candy, 170.3(n)(38)	4.0%	4.0%
Soup mixes, 170.3(n)(40)	3.0%	3.0%
Sugar substitutes,	10.0%	10.0%

170.3(n)(42)		
Sweet sauces, toppings, syrups, 170.3(n)(43)	5.0%	5.0%
White granulated sugar, 170.3(n)(41)	4.0%	4.0%

Basis for GRAS Determination

Enzymotec's GRAS determination for the proposed uses of its OmegaPC™ oil listed in Table 3 is based on scientific procedures as described under 21 CFR 170.30(b). Enzymotec's OmegaPC™ oil has been shown to be substantially equivalent to other edible fish oils (see Table 1), including fish oils that are already GRAS for addition to foods. The estimated intake of OmegaPC™ from the intended uses specified in Table 3, in addition to intakes of EPA and DHA from natural fish oil sources, is safe and is also GRAS under the Federal Food, Drug, and Cosmetic Act). To demonstrate that OmegaPC™ is GRAS under its intended conditions of use, the safety of both whole product intake and EPA+DHA intake from consumption of OmegaPC™ is established under its intended conditions of use, taking into account potential intake of EPA and DHA from natural sources in the diet. Then, this intake of the whole product and of EPA+DHA is determined to be GRAS by showing that the safety of these levels of intake is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, and is based on generally available and accepted information.

The FDA has previously reviewed the safety of consumption of fish oil containing the two omega-3 fatty acids EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS under specified conditions of use (FDA 1997). According to the FDA, the primary safety concerns associated with excessive intakes of EPA and DHA include increased bleeding times, reduced glycemic control among diabetics, and increased levels of low-density lipoprotein (LDL) cholesterol among diabetics and hyperglycemics. To ensure that these safety concerns were mitigated, FDA established a maximum use level of EPA and DHA-containing products of 3 grams/ person/day in all food products for individuals at age 2 years or older. Enzymotec has also conducted a review of the more recent literature post 1997 to determine if more current information pertaining to these safety concerns would contradict what was concluded and recommended in the 1997 FDA opinion regarding EPA and DHA intake from fish oil. This review has focused on the safety of fish oil and of intake of EPA+DHA combined rather than on the distinct metabolic effects of EPA and DHA in isolation. A synopsis of that literature is presented below. Enzymotec did not uncover anything that would contradict FDA's initial safety determination.

FDA has also received and reviewed several GRAS Notices (Table 2) regarding fish oil products containing EPA and DHA as well as GRAS Notices related to EPA and DHA derived from algae and krill. In all of these GRAS Notices, after review, FDA issued a letter informing the notifier that they had "No questions" concerning their conclusion that their

products containing EPA and DHA were GRAS. The fact that FDA has not expressed any concerns with these products coming from different sources and different production processes indicates that FDA is comfortable with the safety of these products. As the information in these GRAS Notices directly pertain to the safety of OmegaPC™, these notices are incorporated by reference into this GRAS Notice.

The publicly available data demonstrating the safety of the proposed uses of OmegaPC™ was reviewed by an Expert Panel convened by EAS on behalf of Enzymotec. This panel evaluated the dietary exposure, source of the substance, method of manufacture, specifications, and contaminant levels, as well as information from recent published toxicological and human studies. The GRAS panel, which Enzymotec regards as qualified by scientific training and experience to evaluate the safety of substances added to food, concluded that OmegaPC™, meeting food grade specifications, are GRAS under their intended conditions of use. Therefore, it is concluded, based on scientific procedures, that the intended use of Enzymotec's OmegaPC™, as shown in Table 3, is safe and is also GRAS.

1.3 Specifications and Identity

Enzymotec has established food grade specifications for the OmegaPC™ product. Compositional analysis and food grade specifications of OmegaPC™ from Enzymotec are presented in Tables 4 and 5, respectively.

The compositional specifications established by Enzymotec address all relevant issues concerning fish oils (Table 4). They address the quality of the oil by providing minimum requirements for fatty acids and phospholipids content, as well as markers of stability and purity. The specifications (Table 5) provide information concerning the fat, and cholesterol contents of OmegaPC™, as well as common contaminants, such as pesticides, dioxins, PAH and heavy metals, which may be present in fish oils. Finally, OmegaPC™'s compositional specifications are similar to those for other fish-derived oils considering phospholipid or omega-3 fatty acid-rich oils and are consistent with the Codex Alimentarius standard for Edible Fats And Oils Not Covered By Individual Standards (CODEX STAN 19-1 981 (Rev. 2-1 999). The analytical procedures employed in the analyses have been validated by a variety of sources as indicated in Table II below. Analytical results from four non-consecutive lots (Appendix I) demonstrate that OmegaPC™ meets the standard specifications.

1.3.1 Product Specifications

Fish meal, the source material for the production of OmegaPC™ is a biomass composed of lipids, sugars and proteins. By using solvent extraction process, the proteins and free sugars are removes so that only lipids are left. Fish meal is derived from multiple edible marine fish species through cooking, pressing and drying of the fish biomass.. The fish species from which the fish meal is produced include anchovies (*Engraulis sp., e.g. Engraulis ringens*), sand eel (*Hyperoplus*

sp., Gymnamodytes sp. or Ammodytes sp., e.g. Ammodytes tobianus), sprat (*Sprattus sprattus*), herring (*Clupea sp., e.g. Clupea harengus*), boar fish (*Capros aper*), Norway pout (*Trisopterus esmarkii*), Capelin (*Malotus villosus*), Blue Whiting (*micromesistius poutassou*) and/or Jack Mackerel (*trachurus murphyi*) , Sardine *(Sardinops sagax)*, Codfish, Haddock, Saithe, menhaden, salmon and other suitable species.

Table 4. Typical Composition of Omega PC™

Parameter	Typical values	Assay method
Phospholipids	39% w/w	^{31}pNMR
Neutral lipids		
Triglycerides	39 % w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Diglycerides	8 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Monoglycerides	1 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Free fatty acids	7 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Other neutral lipids	1 %w/w	AMT-002 "Nonphosphatidyl Lipids Quantification"
Total neutral lipids	55% w/w	
DHA	12 %w/w	USP Monograph "Fish Oil Containing Omega-3 Acids"
EPA	10 %w/w	USP Monograph "Fish Oil Containing Omega-3 Acids"
Cholesterol	24 mg/g	EP 2.4.32
Heavy metals		
Lead	<0.05 ppm	Ph. Eu. Method 2.4.27
Arsenic (total)	16 ppm	Ph. Eu. Method 2.4.27
Arsenic (inorganic)	0.01 ppm	EPA Method 1632 (mod.)
Cadmium	0.01 ppm	Ph. Eu. Method 2.4.27
Mercury	<0.005 ppm	Ph. Eu. Method 2.4.27

Table 5. Product specifications for OmegaPC™

Parameter	Specifications	Assay method
Consistency	Viscous liquid	Visual
Color	Dark brown	Visual
Peroxide value	< 5 meq/Kg	USP 401
Moisture	<4 % w/w	USP 921
Phospholipids	> 35% w/w	^{31}pNMR
DHA+EPA	>18 % w/w	USP Monograph "Fish Oil Containing Omega-3 Acids"
Ethanol residues	<5000 ppm	GC-FID
Hexanes residues	< 5ppm	GC-FID

Microbiological assays		
Total plate count	<1000 cfu/g	Israeli Standard SI 885 Part 3 (1999)
Yeast and Mold	<100 cfu/g	Israeli Standard SI 885 Part 3 (1999)
Molds	<100 cfu/g	Israeli Standard SI 885 Part 3 (1999)
Coliforms	Negative (cfu/g)	USP 61 (2000)
Staphylococcus aureus	Negative (cfu/g)	USP 61 (2000)
Salmonella	Negative (cfu/20g)	Israeli Standard SI 885 Part 3 (1999)
Shelf life	24 months	
cfu=colony forming units		

Although specifications were not established for polyaromatic hydrocarbons (PAH), analyses were conducted for them as we aware of concerns associated with the presence of these compounds. None were present at levels of toxicological concern. It should be noted that PAH contamination is generally not considered to be an issue for standard fish oils although it might be a problem in smoked fish products.

1.4. Manufacturing Process

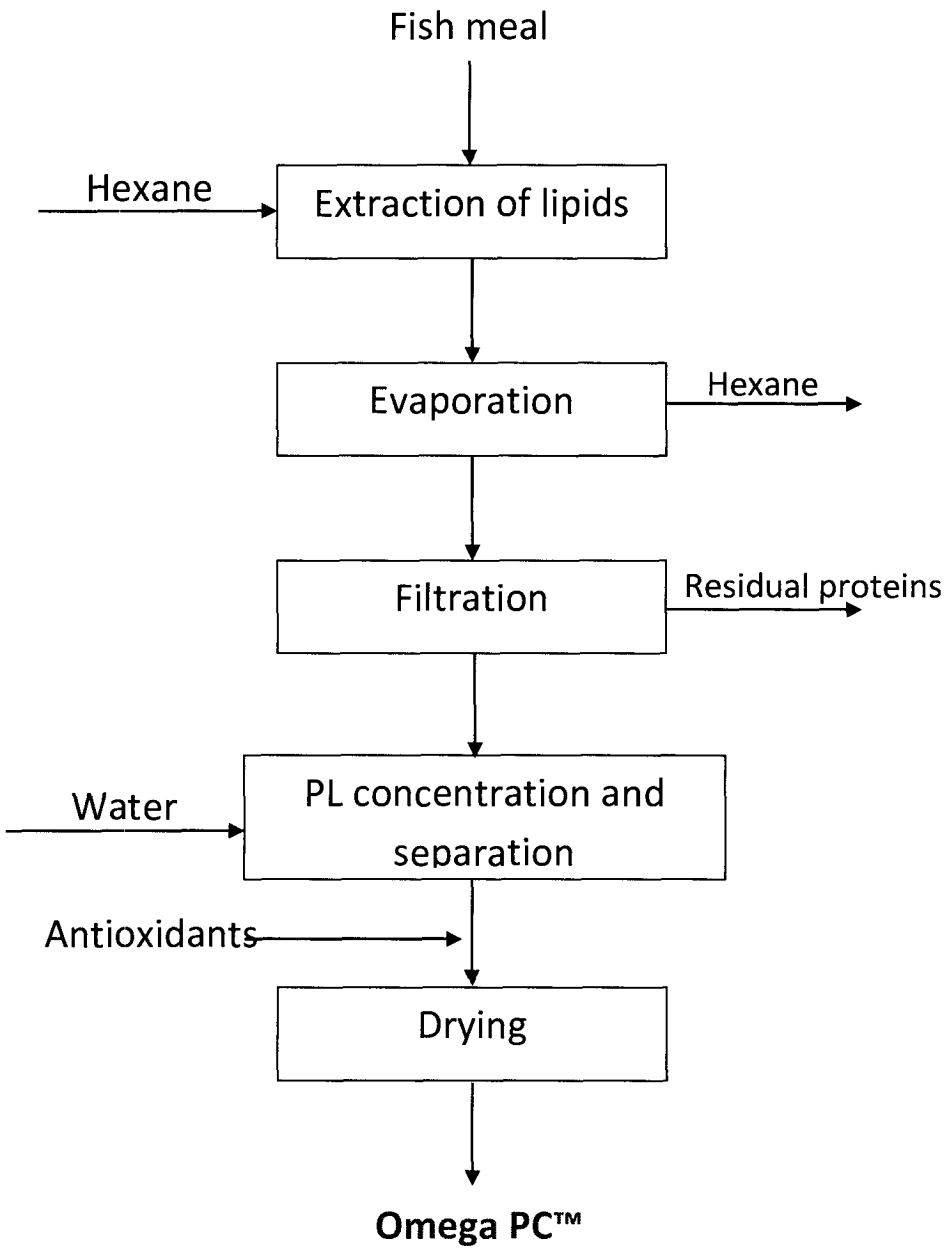
1. Overview

OmegaPC™ is produced through solvent extraction of fish meal.. Fish meal, a biomass composed of lipids, sugars and proteins, is generally produced from fresh or frozen fish by cooking followed by separation into a solid and a liquid phase, usually by pressing. The solid phase is further dried in an industrial dryer to a moisture content of 5-15% to produce the final fish meal. The fish meal is inspected for acceptability prior to being extracted.

Lipids from the fish meal are extracted continuously using hexane meeting the specifications in the Food Chemicals Codex, 5th Ed. Following the solvent extraction process, the liquid organic phase, which contains the solvent and the extracted lipids, undergoes vacuum evaporation in order to remove the solvent. The crude oil, which contains phospholipids and triglycerides, is then filtered in order to remove residual proteins and other impurities. Following the filtration stage, the phospholipids are concentrated by mixing the crude oil with water and subjecting it to centrifugation to provide a phospholipid-rich phase (the crude product) and a phospholipid-poor phase (fish oil). The phospholipid-rich phase is dried from residual water by vacuum evaporation and may further be mixed with fish oil for standardization . Food grade antioxidants are then added to the product in accordance with good manufacturing practice. The process is conducted in a nitrogen-rich environment in order to maintain the stability of the product throughout the production process.

Analyses of four non-consecutive batches (Appendix I) demonstrate that the manufacturing process results in product that consistently meets product specifications. An overview of the manufacturing process for Omega PC™ is shown below.

Figure 1: Process Flow Diagram



1.5. Current Uses

Enzymotec intends to market its OmegaPC™ product for addition to several categories of foods as a nutrient supplement (2 1 CFR 170.3(o)(20)) to increase the dietary intake of the omega-3 fatty acids EPA and DHA. The food categories proposed for addition and the proposed addition levels are listed in Table 3. These are the same food categories as are specified in the GRAS regulation for menhaden oil (21 CFR 184.1472(a)(3)), and OmegaPC™ thus serves as an alternative to menhaden oil as a source of EPA and DHA. OmegaPC™ **is** proposed for addition at the same use levels proposed for menhaden oil (also shown in Table 3), reflecting the average 22% EPA+DHA composition of OmegaPC™ compared with 22% EPA and DHA in menhaden oil. Thus, the addition rates of EPA+DHA are the same for OmegaPC™ as for menhaden oil.

It is intended that OmegaPC™ will be used as the sole added source of EPA and DHA in any given food category and is not to be combined or augmented with any other source of EPA or DHA in making a food product. Therefore, the overall consumer exposure to EPA and DHA will not change as OmegaPC™ is expected to be a substitute for menhaden oil and other EPA/DHA products.

1.6. Regulatory Status

Enzymotec has determined that OmegaPC™ is GRAS for use in food as described in this document.

1.7. Technical Effects

The intended use of OmegaPC™ is as a nutrient supplement (21CFR 170.3(o)(20) in the same foods as listed under 21 CFR 184.1472.

1.8. Intended Use Levels and Food Categories.

Enzymotec intends to market its OmegaPC™ product for addition to several categories of foods as a nutrient supplement (2 1 CFR 170.3(o)(20)) to increase the dietary intake of the omega-3 fatty acids EPA and DHA. The food categories proposed for addition and the proposed addition levels are listed in Table 3. These are the same food categories as are specified in the GRAS regulation for menhaden oil (21 CFR 184.1472(a)(3)), and OmegaPC™ thus serves as an alternative to menhaden oil as a source of EPA and DHA. OmegaPC™ **is** proposed for addition at the same use levels proposed for menhaden oil (also shown in Table 3), reflecting the average 22% EPA+DHA composition of OmegaPC™ compared with 22% EPA and DHA in menhaden oil. Thus, the addition rates of EPA+DHA are the same for OmegaPC™ as for menhaden oil.

1.8.1. Estimated Daily Intake from the Intended Uses

It is intended that OmegaPC™ will be used as the sole added source of EPA and DHA in any given food category and is not to be combined or augmented with any other source of EPA or DHA in making a food product. Therefore, the overall consumer exposure to EPA and DHA will not change as OmegaPC™ is expected to be a substitute for menhaden oil and other EPA/DHA products.

2. REVIEW OF SAFETY DATA

Introduction

The FDA has previously reviewed the safety of consumption of fish oil containing the two omega-3 fatty acids EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS under specified conditions of use (FDA 1997). According to the FDA, the primary safety concerns associated with excessive intakes of EPA and DHA include increased bleeding times, reduced glycemic control among diabetics, and increased levels of low-density lipoprotein (LDL) cholesterol among diabetics and hyperglycemics. The FDA examined the scientific documentation for these health concerns and found that there appeared to be no statistically relevant risks as long as the consumption of fish oil is limited to 3 g/p/d of EPA and DHA. Enzymotec has reviewed the more recent literature to determine if more current information pertaining to these safety concerns would contradict what was concluded and recommended in the 1997 FDA opinion regarding EPA and DHA intake from fish oil. This review has focused on the safety of fish oil and of intake of EPA+DHA combined rather than on the distinct metabolic effects of EPA and DHA in isolation. Enzymotec did not find any information that would contradict FDA's earlier conclusion. Nonetheless, a synopsis of the resultant literature search performed by Enzymotec is given below to establish that the safety of OmegaPC™ is not in question.

The safety of omega-3 fatty acids is supported by their long history of ingestion as a component of the human diet and a large number of clinical trials investigating their effects. Although adverse effects, namely elevated LDL-cholesterol levels, prolonged bleeding time, and effects on glycemic control have been reported in some subpopulations, the available data indicate that intakes of up to 3 g of DHA + EPA/person/day pose no significant risk on these parameters. In its 1997 menhaden oil decision, the FDA concluded that combined consumption of up to 3 g of DHA + EPA/person/day poses no significant risk for bleeding time, produces no clinically significant effect on glycemic control, and is safe with respect to the effect on LDL cholesterol (FDA, 1997). In its final rule affirming menhaden oil as GRAS, the FDA (2005) published maximum use-levels to ensure that the total daily intake of combined DHA and EPA does not exceed 3.0 g/person.
Enzymotec is also aware that a Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid

(DPA) has recently been published by the European Food Safety Authority (EFSA)[3]. EFSA concluded that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for adults.

Although the high LCPUFA doses used in animal studies may produce adaptive non-specific effects in liver metabolism and histomorphology, these effects are related to the extra metabolic workload and are not relevant to exposure at much lower doses.

Adverse effects associated with omega-3 polyunsaturated fatty acid consumption have been reported in a few special subpopulations (IOM, 2005). It has been suggested that individuals exhibiting glucose intolerance or diabetic conditions use caution with omega-3 fatty acids as some have required increased doses of hypoglycemic agents (Glauber et al., 1988; Kasim et al., 1988; Friday et al., 1989; Zambon et al., 1992).

The results of numerous clinical studies published since the FDA review in 1993 indicate the DHA provided in fish or marine-derived oils at levels up to 6 g DHA/person/day would not be expected to produce adverse effects on these parameters. These results are consistent with the FDA conclusion in its 1997 menhaden oil decision that the combined consumption of up to 3 g of DHA + EPA/person/day poses no significant risk for bleeding time, produces no clinically significant effect on glycemic control, and is safe with respect to the effect on LDL cholesterol (FDA, 1997).

The US Department of Health and Human Services Agency for Healthcare Research and Quality (2004) identified 148 studies on omega-3 fatty acids that evaluated over 20,000 subjects for adverse effects. The most common side effects were gastrointestinal complaint, reported among 6.6% of patients taking omega-3s versus 4.3% in placebo groups. An increased incidence of bleeding was not observed, and only 1 of the 148 studies reported such an association in patients randomized to 6 g/day of omega-3. There were no reported deaths or life-threatening illnesses as a consequence of omega-3 consumption. No adverse effects were reported in 77 of the 148 studies. Based on this review, the agency concluded that adverse effects related to consumption of fish oil or a-linolenic supplements appear to be minor.

2.1 Animal studies

Enzymotec also reviewed a number of animal studies that were uncovered since the FDA 1997 report. The results of these studies did not point to any new safety concerns. These studies were tabulated and summarized (see Appendix II).

2.1.1 Metabolism of phosphatidylcholine

It is well established and recognized that phosphatidylcholine (lecithin) from either plant or animal sources is handled the same metabolically. Lecithin is absorbed into the mucosal cells of the small intestine, mainly in the duodenum and upper jejunum, following digestion by the

[3] Available at http://www.efsa.europa.eu/en/efsajournal/doc/2815.pdf.

pancreatic enzyme phospholipase A2 (Arnesjo ***et al.,*** 1969; Belleville and Clement, 1969), by which the fatty acids in the 2 position are hydrolyzed to form lysophosphatidylcholine (Nieuwenhuizen ***et al.,*** 1974). Following absorption by the enterocytes, reacylation of lysophosphatidylcholine takes place in these intestinal mucosal cells, reforming phosphatidylcholine, while the previously released fatty acids can be further used for triglyceride synthesis (Tso and Fujimoto, 1994). Phosphatidylcholine is then transported by the lymphatic system in the form of chylomicrons to the blood and metabolized by peripheral tissues. After the liver takes up the chylomicron remnants, the lipids are repackaged and secreted in the very low density lipoproteins (VLDL) (Ginsberg, 1998; Kang and Davis, 2000). Phosphatidylcholine is also incorporated into cell membranes, particularly in the lung. Phosphatidylcholine is also metabolized to choline, fatty acids and glycerol. The fatty acids and glycerol are either oxidized to produce energy or become involved in lipogenesis. Choline serves as a precursor of the neurotransmitter acetylcholine and serum choline levels have been shown to peak between 2 to 6 hours after oral intake of phosphatidylcholine.

2.2 Human Studies involving marine-based phospholipids

The clinical database of marine-based phospholipids includes 16 clinical trials, most of which have been identified as double-blind protocols. The majority of these studies used krill oil as a source of DHA and EPA that combines both phospholipids and glycerides, as in the case of OmegaPC. The compositional similarity of OmegaPC™ to Krill oil (see comparison in Appendix III) justifies the use of krill oil studies to support the safety of OmegaPC™. Although the investigations were designed to study the efficacy of the tested articles, clinical observations also included any adverse effects.

In a preliminary study (Dupont, 2006), thirty patients with all types of psoriasis received 400 mg of marine phospholipids of wild pelagic fish extracts per day for a period of 4-6 months. The tested article was composed of 45% PC, 29% PE, 16% PI, 5% PS and 5% sphingomyelin. The fatty acids esterified to the phospholipids were mainly DHA (60%), EPA (30%) and DPA (3%). No adverse events were reported.

In another open label trial (Taylor 2010), thirty-one tumor patients with various tumor entities suffering from weight loss received 1.5g/d of marine phospholipids (source not specified) for a period of 6 weeks. Compliance, body weight, appetite, and quality of life as well as the fatty acid profile in plasma and blood cells were monitored. Marine phospholipids were very well accepted and no treatment- related adverse events were reported.

In clinical trials conducted with krill oil, over 1000 subjects participated and the treatment lasted for periods of up to 6 months. The doses used in these trials ranged from 300mg to 4g/day. Based on the data provided in GRAS notices submitted for the tested products (GRN Nos. 242 and 371), these levels of krill oil provided between 135mg and 1.8g omega-3 enriched phospholipids and between 120mg and 1.6g omega-3 enriched glycerides. Results from these studies show that oral administration of a combination of marine phospholipids and glycerides at doses of up to

4g/day for up to 12 weeks were without any significant adverse effects. In the largest double-blind, placebo-controlled trial (Berge et al., 2013), only three participants of the 300 withdrew from the study, none due to treatment-related issues. Safety assessments included measurements of blood pressure, pulse rate, body temperature, and the collection of information on unsolicited adverse events at all visits, as well as 12-lead echocardiogram (ECG), physical checkup, urinalysis, hematology and clinical chemistry at the screening and end-of-study visits. Overall, krill oil supplementation was well tolerated in all groups and no serious adverse events related to study product occurred during the study. There were two subjects with unrelated serious adverse events, including asthma and cellulitis. Other incidences of non-serious adverse events that could possibly be related to study product intake were: hypertension (1), soft stool (2), flatulence (1), upset stomach (3), gastrointestinal discomfort (1), decreased appetite (1), headache (1), taste change (1), diarrhea (4), fishy burps (1), heartburn (1) and intermittent belching (1). Body weight and blood pressure remained unchanged during the 12-week study compared to baseline values in all study groups.

A summary of clinical trials involving marine-based phospholipids, including designs, doses and adverse effects noted in these investigations is presented in Table 6.

Table 6. Reported adverse effects of marine-based phospholipids in clinical trials

Reference; study design	PL Source	Number Subjects	Dose (mg/day); Duration	Adverse Effects Reported
Banni et al., 2011; DB-PC	Krill	63	2g/d; 4w	No symptoms of adverse reactions were reported
Berge 2013; OL	Krill	12	4g/d; 24w	No symptoms of adverse reactions were reported
Berge 2013; DB-PC	Krill	300	0.5-4.0g/d; 12w	No significant adverse events reported. Several incidences of non-serious adverse events.
Bunea et al., 2004 ; DB	Krill	120	1-3 g/day (BMI-dependent); 12w	No symptoms of adverse reactions were reported
Dupont, 2006	Wild pelagic fish	30	400mg/d; 4-6 m	No symptoms of adverse reactions were reported

Reference; study design	PL Source	Number Subjects	Dose (mg/day); Duration	Adverse Effects Reported
Deutsch et al., 2007; DB-PC	Krill	90	300mg; 30 days	No symptoms of adverse reactions were reported
Hayashi 1999; OL	Salmon roe	6	1.6g/d; 6m	No symptoms of adverse reactions were reported
Konagai 2013; DB	Krill	45	Equivalent to 300 mg/d EPA+DHA	No symptoms of adverse reactions were reported
Maki et al, 2009; DB-PC	Krill	76	2g/d; 4w	No symptoms of adverse reactions were reported
Ramprasath 2013; DB-PC	Krill	24	3g/d; 4w	No symptoms of adverse reactions were reported
Sampalis, 2003; DB-PC	Krill	70	2g KO/d; 3m	No SAE were reported
Schuchardt et al., 2011; DB-PC	Krill	12	Equivalent to 1680 mg of n-3; 72 h	No symptoms of adverse reactions were reported
Skarpańska et al., 2010; DB	Krill	17	500mg; 6m	None reported
Taylor 2010; OL	Marine (exact source not specified)	31	1.5g/d; 6m	No symptoms of adverse reactions were reported
Trepanowski et al., 2012; DB	Krill	39	2g/d; 4w	No symptoms of adverse reactions were reported
Ulven et al., 2011; OL	Krill	113	3g/d; 7w	No symptoms of adverse reactions were reported
Wakeman 2013; OL	Krill	29	350mg/d in combination with thiamine HCl (1.4 mg), riboflavin (1.6 mg), pyridoxine HCl (2 mg), soy isoflavones (50 mg;),	One subject reported "cramps" which were resolved and not clearly related to supplement.

Reference; study design	PL Source	Number Subjects	Dose (mg/day); Duration	Adverse Effects Reported
			rosemary extract (50 mg; 3m	
DB = double-blind; PC= placebo-controlled; OL=Open label; d=day; w = weeks; m = months				

2.3 Pre-clinical studies involving marine-based phospholipids

Rosmeisl et al (2012) compared the effects of omega-3 bound to phospholipids to those of omega-3 bound to triglycerides, both derived from fish, in an animal model of obese-related disorders. In that study, male C57BL/6J mice were weaned on a standard Chow until study initiation. To induce obesity, three-month-old mice were assigned to high fat (HF) diet (lipids, 35% wt/wt; mostly corn oil; virtually DHA/EPA-free). The study consisted of three parts: first, a 'prevention study' was performed to characterize the effects of LC n-3 PUFA on the development of obese phenotype, while replacing part of corn oil in the HF diet with omega-3 either as triglycerides (DHA, 46% wt/wt; EPA, 14% wt/wt) or as phospholipids from marine fish (DHA, 17–20% wt/wt; EPA, 5–8% wt/wt) in order to achieve a sum of DHA and EPA (DHA/EPA) of 30 g per kg diet. A group of mice was also treated using a HF+omega-3-PL diet containing 10 g DHA/EPA per kg diet. This part of the study lasted for a period of 9 weeks. Effect of the added omega-3 on obesity induced adverse consequences, such as weight gain, lipid profile and glycemic control, were tested. While the HF+omega-3-TG treatment mainly decreased plasma non-esterified fatty acid (NEFA) levels, the HF+omega-3-PL treatment showed a strong tendency to lower body weight gain and to reduce adiposity and adipocyte size, as well as exerting significant hypolipidemic effects. Importantly, glucose tolerance was only improved in response to the HF+omega-3-PL treatment. Only the cHF+omega-3-PL treatment resulted in lower lipid accumulation in the liver at both dietary DHA/EPA concentrations.

Secondly, a 'bioavailability study' was performed that was similar to that described above, except that the dietary treatments lasted for only two weeks. In the prevention study, plasma concentrations of both DHA and EPA were higher in the HF+omega-3-PL treated mice than in the HF+omega-3-TG treated mice. At the tissue level, DHA as well as EPA concentrations in the triglyceride fraction either from the liver or total white adipose tissue lipids did not differ between the treatments. However, EPA was enriched in hepatic phospholipids of the HF+omega-3-PL treated mice.

Thirdly, in a 'reversal study', obesity was induced by HF diet feeding between three and seven months of age prior to 9-weeklong treatment using HF+omega-3-TG or HF+omega-3-PL diets

supplemented with 30 g DHA/EPA per kg diet. To mimic a typical situation in overweight or obese, insulin resistant type 2 diabetic subjects, all diets were also supplemented by metformin (2 g per kg). HF+omega-3-TG and HF+omega3-PL treatments both decreased weight gain, with a stronger effect being exerted by the HF+omega-3-PL treatment. Both treatments reduced adiposity to a similar extent as well as the levels of plasma lipids; they suppressed glycemia and tended to improve glucose tolerance.

In a second study by the same group, Rossmeisl et al (2013) report on the effects of marine PLs on hepatic steatosis. In that study, possible mechanisms leading to the beneficial effects of omega-3 PLs on hepatosteatosis was tested. C57BL/6N mice were fed for 7 weeks an obesogenic high-fat (HF) diet or HF diet supplemented with PC-rich concentrate from herring (replacing 10% of dietary lipids), a HF diet containing rosiglitazone (10 mg/kg diet), or herring PC + rosiglitazone. Metabolic analyses, hepatic gene expression and lipidome profiling were performed. Results showed that herring PC and herring PC + rosiglitazone prevented HF diet induced weight gain and glucose intolerance, while all interventions reduced abdominal fat and plasma TGs. Herring PC and herring PC + rosiglitazone also lowered hepatic and plasma cholesterol and reduced hepatosteatosis. Microarray analysis revealed integrated downregulation of hepatic lipogenic and cholesterol biosynthesis pathways by herring PC, while rosiglitazone - induced lipogenesis was fully counteracted in herring PC + rosiglitazone. Gene expression changes in herring PC group and in herring PC + rosiglitazone group were associated with preferential enrichment of hepatic PC and PE by DHA/EPA.

A recent study tested the effects of omega-3 PLs from marine sources on obesity-related metabolic disorders. Liu et al (2013) report that EPA-PL [from sea cucumber (*Cucumaria frondosa*] and DHA-PL [from squid *(Sthenoteuthis oualaniensis)* eggs] were administered to high fat (HF) diet-induced obese C57BL/6J mice for 8 weeks. DHA-PL and EPA-PL significantly decreased adipose tissue weight, reduced blood pressure and lowered serum and hepatic TG levels. Serum insulin, MCP-1 and IL-6 levels were also efficiently reduced by treatment with DHA-PL and EPA-PL. The anti-obesity and lipid-lowering effects of EPA-PL were superior to DHA-PL, while DHA-PL exhibited better anti-hypertension effects than EPA-PL. The effects of DHA-PL and EPA-PL on glucose intolerance and inflammation were basically equivalent. DHA-PL and EPA-PL up-regulated genes involved in insulin-sensitizing actions in the adipose tissue and suppressed hepatic SREBP-1c mediated lipogenesis. EPA-PL also significantly activated PPARα mediated fatty acid β-oxidation in the liver.

3. SUMMARY

Safety of EPA and DHA

Typically, EPA and DHA are contained in oily fish, such as salmon, lake trout, tuna and herring. The composition of EPA and DHA in OmegaPC™, the subject of this notification, is about 10% w/w and 12% w/w, respectively. The average total of EPA+DHA in OmegaPC™ is

22%. The safety of DHA and EPA, the principal fatty acids in OmegaPC™, has been extensively evaluated by the FDA in the 1997 final rule on the GRAS affirmed use of menhaden oil as a direct food ingredient and also regarding the use of omega-3 fatty acids as a dietary supplement in 2005. In 1997, menhaden oil was affirmed as GRAS by FDA as a direct human food ingredient with specific limitations of use to ensure that the total daily intake of EPA and DHA would not exceed 3 g/person/day (62 FR 30751; June 5, 1997; 21 CFR 184.1472). Because of concerns over possible adverse effects of fish oil consumption on bleeding coagulation time, glycemic control, and LDL cholesterol, FDA established maximum use levels of menhaden oil in certain foods (62 FR 30751 at 30757; June 5, 1997; amended March 23, 2005; 70 FR 14531). FDA reaffirmed that the intake of DHA and EPA must not exceed 3.0 g/day from all fish oil sources and in doing so, FDA placed specific limitations, including the category of foods, the functional use of the ingredient, and the level of use, to ensure that the consumption of EPA and DHA would not exceed 3.0 g/day.

In addition, FDA has not objected to certain GRAS notifications for additional sources of EPA and DHA as food ingredients (fish oils other than menhaden oil, micro-algal oil and a yeast oil) (GRAS Notification Nos. GRN 105, GRN 109, GRN 138; GRN 146; GRN 193; GRN 200, GRN 319, GRN 355). These GRAS Notifications proposed maximum use levels consistent with those specified in the final rule affirming as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use. FDA has also responded without objection to a GRAS notification from Martek Biosciences Corporation for high DHA algal oil DHA. Martek estimated that the use of algal oil in a number of food categories at the maximum proposed use levels would result in a mean exposure of no more than 1.5 g DHA/day (GRAS Notice No. GRN 137). Additionally, the FDA responded without objection to 3 GRAS notifications related to krill oil. Enzymotec proposed a number of food categories that have already been described in 21 CFR 184.1472. The maximum levels of addition were calculated so as not to exceed the upper limit of 3 g/day of DHA and EPA as outlined in the menhaden oil regulation (GRN 000226). Neptune estimated that the use of krill oil in a number of food categories would result in a maximum daily consumption of EPA and DHA of 2.2 g/p/d (GRN 242). Aker Biomarine estimated that based on the 90th percentile EDI for krill oil, the combined maximum EDI for EPA and DHA would be 1.95 g/p/d (GRN 371).

FDA has also responded without objection to a GRAS notification on algal oil DHA from Martek Biosciences Corporation. Martek estimated that the use of algal oil in a number of food categories at the maximum proposed use levels would result in a mean exposure of no more than 1.5 grams of DHA per day (GRAS Notice No. GRN 000137).

The Expert Panel members are aware of these GRAS Notices and has considered them in their deliberations.

OmegaPC™, the subject of this safety assessment is a fish-based lipid extract mainly comprised of phospholipids and triglycerides containing primarily of 22% EPA+DHA. FDA affirmed the

GRAS status of menhaden oil under 21 CFR 184.1472 and established that a daily intake of EPA and DHA combined not exceed 3 grams per person per day is safe. The scientific basis to support the establishment of this safe level of intake was published in the Federal Register at page 30751 (62, FR 30551; June 5, 1997), as part of the final rule on menhaden oil. As reported above, menhaden oil is one of the key edible fish sources that have a high concentration of EPA and DHA. Menhaden oil is a refined marine oil that is derived from menhaden fish *(Brevoortia* species) which are any of a general species of valuable Atlantic coastal fishes. It consists primarily of fatty acid triglycerides, with small amounts of monoglycerides and diglycerides. The triglycerides are esters of glycerol and fatty acids with chains of 14 to 22 carbon atoms. Menhaden oil differs from edible vegetable oils and animal fats in its high proportion of polyunsaturated fatty acids with 4, 5 and 6 double bonds (about 25 percent). The mean percentages for these polyunsaturated fatty acids in menhaden oil are C18:4 (2.3 percent), C20:4 (2.0 percent), C20:5 (13.1 percent), C22:5 (2.5 percent) and C22:6 (6.7 percent). Note that C20:5 and C22:6 are EPA and DHA, respectively, and are the major source of omega-3 fatty acids from fish oil. Menhaden oil also contains about 33 percent saturated fatty acids and about 31 percent monounsaturated fatty acids. In their review, FDA identified three potential safety issues of menhaden oil consumption above 3 grams per person per day: increased bleeding time by reducing platelet aggregability, a potential concern about glycemic control in non-insulin-dependent diabetics, and potential increases in LDL cholesterol.

There have been many published studies evaluating the safety of omega-3 fatty acids. These have all been evaluated by FDA in arriving at the determination that menhaden oil and its component fatty acids including EPA and DHA are GRAS as food ingredients subject to the limitations in 2 1 CFR 184.1472 and the final rule affirming as Generally Recognized as Safe (GRAS) menhaden oil (March 23, 2005; 70 FR 14530). FDA also permitted the use of a Qualified Health Claim on dietary supplements containing EPA and DHA on October 31, 2000 as well as for conventional foods on September 8, 2004. In the October 31, 2000 letter, FDA concluded that the use of EPA and DHA omega-3 fatty acids as dietary supplements is safe and lawful under 21 CFR 101.14, provided that daily intakes of EPA and DHA omega-3 fatty acids do not exceed 3 g/p/d from conventional food and dietary supplement sources. Further, FDA concluded that in order to help ensure that a consumer does not exceed an intake of 3 g/p/d of EPA and DHA omega-3 fatty acids from consumption of a dietary supplement with the qualified claim, an EPA and DHA omega-3 fatty acid dietary supplement bearing a qualified claim should not recommend or suggest in its labeling, or under ordinary conditions of use, a daily intake exceeding 2 grams EPA and DHA.

The production process for OmegaPC™, summarized above, is a process that is similar in many respects to the standard industry practice for the processing of fish oils. Furthermore, this process is well characterized and can consistently yield a food-grade product that is safe for human consumption with the ongoing analytical testing and quality control procedures typically performed by this industry. Acceptable analytical methodology is employed for OmegaPC™ that include measurement of oxidative by-products (acid value, peroxide valuee), PCDDs and PCDFs, PCBs, PAH and heavy metals including lead, cadmium, mercury, and arsenic. Enzymotec will be sampling and analyzing their OmegaPC™ product and process to ensure compliance with the specifications shown in Table 5.

The fish-based lipid extract that is the subject of this safety assessment is comprised primarily of omega-3 fatty acids (EPA + DHA) bound to phospholipids and triglycerides. It is known by the commercial name of OmegaPC™. The intended applications of this fish-based lipid extract product will be for the same uses in foods for which menhaden oil is permitted under 21 CFR 184.1472 and as noted in the final rule (70 FR 14530 – 14532; March 23, 2005). The maximum levels of use will be the same as those provided by menhaden oil based on EPA+DHA content of 22 percent in menhaden oil and 22 percent in OmegaPC™. These proposed uses are presented in Table 3. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above (See Table 2) including one for a marine oil concentrate. Because the combined EPA and DHA content of foods to which OmegaPC™ will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule and 21 CFR 184.1472, OmegaPC™ will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of OmegaPC™.

From the foregoing analysis and rulemaking decisions of FDA on the GRAS affirmation of menhaden oil and of EPA and DHA, as well as on the submitted GRAS Notices where the agency had no objection to the conclusions of being GRAS including a specific submission on an omega-3 fish oil concentrate, Enzymotec's OmegaPC™ is considered GRAS for the proposed uses specified in the regulations under the conditions described and at the maximum use levels described in Table 3.

In addition, a search of the recent scientific literature was conducted to determine if there were any new publications relating to the safety of EPA and DHA since FDA's final regulation on the GRAS affirmation of menhaden oil. A review of the pertinent articles uncovered is discussed in this document. No new safety issues were identified.

The safety of consumption of OmegaPC™ used as an ingredient in food is based on its similarity to currently marketed fish oil products that have been the subject of several GRAS Notices referenced in this document, as well as the safety of ingestion of its major constituents, EPA and DHA. The safety of consumption of the whole product was determined by evaluating the source of the product, the production process, the nature and quantity of impurities, product specifications, and the identity and positional distributions of EPA and DHA in the glycerides comprising the product. Appropriate product specifications have been established to ensure that the final product is food grade, and compositional analysis of the product supports the presumption that there is no toxicological concern from any product impurities. Further, as long as the OmegaPC™ use is in the food categories identified above at a level of that is consistent with the maximum permissible levels of menhaden oil, and the resulting mean potential intake is less than 3.0 grams per day of EPA and DHA combined, it is safe, and GRAS , for addition to food.

4. CONCLUSION

Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively concluded that OmegaPC™, a fish-based lipid extract containing EPA and DHA, meeting the specifications cited above, and produced as described, is GRAS when used as a nutrient supplement (21 CFR 170.3(o)(20) in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation (21 CFR 184.1472) when used at a levels equivalent to that of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that OmegaPC™, a fish-based lipid extract containing EPA and DHA, when used as described, is GRAS based on scientific procedures.

(b) (6)

Stanley M. Tarka, Jr., Ph.D. — 01 August 2014 — Date

(b) (6)

Sigalit Zchut, Ph.D. — 07-24-14 — Date

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. — July 30, 2014 — Date

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Appendix I. Analytical data from four different manufacturing lots

Parameter	Typical Level/specifications	Lot (b) (b)	Lot (b) (b)	Lot (b) - (b)	Lot (b) (6)
Phospholipids	>35 %w/w	39.26	39.22	38.97	38.19
Neutral lipids					
Triglycerides	41 % w/w	40.5	41.0	41.7	34.3
Diglycerides	7 %w/w	7.2	7.3	7.2	8.3
Monoglycerides	1 %w/w	0.5	0.7	0.6	0.5
Free fatty acids	7 %w/w	6.9	7.1	6.3	6.8
Other neutral lipids	1 %w/w	0.9	0.7	0.7	0.3
Total neutral lipids	<65% w/w	56.0	56.8	56.6	50.2
DHA	>18 %w/w (DHA+EPA)	11.98	11.91	11.91	8.86
EPA		10.87	10.92	10.92	10.11
Cholesterol	23 mg/kg	23.2	22.1	22.6	26.84
Peroxide value	<5 meq/kg	<0.2	<0.2	<0.2	1.85
Moisture	<4.0 % w/w	0.34	0.41	0.56	3.57
Saponification value	205mg KOH/g	199.62	205.90	210.34	168.58
Iodine value	165 gI2/100g	156.70	156.97	194.38	149.30

Heavy metal analysis from three manufacturing lots

	Lot (b) (6)	Lot (b) (6)	Lot (b) (6)	Lot (b) (6)
Lead (ppm)	<0.05	<0.05	<0.05	<0.05
Arsenic (total) (ppm)	17	17	17	13
Arsenic (inorganic) (ppm)	0.011	0.010	0.008	0.012
Cadmium (ppm)	<0.01	0.01	0.01	<0.01
Mercury (ppm)	<0.005	<0.005	<0.005	<0.005

Ethanol and hexanes residues from three manufacturing lots

	Lot (b) (6)	Lot (b) (6)	Lot (b) (6)	
Residual ethanol (ppm)	<50	<50	<50	
Residual hexanes (ppm)	1.54	1.65	1.78	

Dioxins and Dioxin-like PCBs[1]

	Lot (b) (6)	Lot (b) (6)	Lot (b) (6)	Lot (b) (6)
Dioxins and Furans (pg/g WHO-PCDD/F TEQ)	0.137	0.096	0.115	0.029
Sum of Dioxins & dioxin-like PCBs (pg/g WHO-PCDD/F+PCB-TEQ)	1.087	0.989	1.029	0.503
Dioxin-like PCBS (pg/g WHO-PCB TEQ)	0.95	0.893	0.914	0.593
Total PCBs 6 (ng/g)	4.77	4.17	4.52	1.22

[1]Upperbound concentrations calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification

PAH

	Lot (b) (6)	Lot (b) (6)	Lot (b) (6)	Lot (b) (6)
Benzo(a)anthracene (µg/kg)	<0.5	<0.5	<0.5	0.56
Chrysene (µg/kg)	<0.5	<0.5	<0.5	0.64
Benzo(b)fluoranthene (µg/kg)	<0.5	<0.5	<0.5	0.66
Benzo(k)fluoranthene (µg/kg)	<0.5	<0.5	<0.5	<0.5
Benzo-(j)-fluoranthene (µg/kg)	<0.5	<0.5	<0.5	<0.5
Benzo(a)pyrene (µg/kg)	<0.5	<0.5	<0.5	<0.5
Indeno(1,2,3-cd)pyrene (µg/kg)	<0.5	<0.5	<0.5	<0.5
Dibenzo(a,h)pyrene (µg/kg)	<1	<1	<1	<1
Benzo(ghi)perylene (µg/kg)	<0.5	<0.5	<0.5	<0.5
Dibenzo(a,l)pyrene (µg/kg)	<1	<1	<1	<1
Dibenzo(a,i)pyrene (µg/kg)	<1	<1	<1	<1
Dibenzo(a,h)anthracene (µg/kg)	<0.5	<0.5	<0.5	<0.5
Dibenzo(a,e)pyrene (µg/kg)	<1	<1	<1	<1
Cyclopenta(c,d)pyrene (µg/kg)	<1	<1	<1	<1
5-Methylchrysene (µg/kg)	<1	<1	<1	<1
Benzo-(c)-flourene (µg/kg)	<1	<1	<1	<1

APPENDIX II. Summary of Pre-Clinical studies Evaluating the Effect of Marine Phospholipid Oil

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Ruggiero-Lopez 1994 [1]	18 male Sprague-Dawley rats, from d19 till d 22	3 d	Standard diet supplemented with corn oil, menhaden oil or krill oil each @ 10% of diet	Effect of krill oil (KO) and fish oil (FO) on intestinal fucosylation process at weaning, a means to demonstrate the lack of krill toxicity	KO diet was very well tolerated and induced a slight modification in fucose and mannose proportions in intestinal glycoprotein sugars.		"The results confirm the harmlessness of krill derived products and their possible use in human nutrition"
Venkatraman 1994 [2]	60 weanling B/W female mice	6 m	Diet containing either corn oil, fish oil or krill oil (10% wt)	To determine whether the protective action of n-3 lipids is mediated through their antioxidant defense system.	"The data indicate that one of the mechanisms through which the n-3 lipids delay the onset of autoimmune diseases in B/W mice may be through maintenance of higher activities and expression of hepatic antioxidant enzymes"	"Additional studies are required to clarify the exact role of specific lipids and the levels that would affect antioxidant enzyme mRNA levels"	"Our data indicate that a diet containing marine lipids with very long-chain n-3 fatty acids may delay the onset of autoimmune disease in mice".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Tanaka 2000 – article in Japanese and inaccessible. Data from abstract only [3]	Mice	Unknown	1) Tuna oil (30% DHA) 2) tridocosahexa-enoylglycerol 3) Free fatty acid DHA 4) ethylester-DHA 5) di-DHA-phatidylcholine 6) Phospholipid extracted from fish roe.	To study the effect of various molecular derivatives of DHA (TG, EE etc.) on anti-inflammatory function			"These results suggest that structural modifications of DHA may influence its anti-inflammatory actions. The authors have shown that different molecular derivatives of DHA affected its anti-inflammatory function"
Zhu 2008 [4]	*60 adult male SD rats * Human colon cancer cells, SW480	7 w	16.65 g/L, 33.3 g/L, 99.9 g/L and 199.8 g/L of krill oil	To evaluate the effect of KO on serum lipids of hyperlipidemic rats and human colon cancer cells (SW480).	Total cholesterol, LDL cholesterol and serum triglycerides were reduced following KO intake while HDL cholesterol increased.	"The mechanism of the KO used in the present study and the relative contributions of its components requires further study".	"Our findings indicated that the consumption of KO may provide benefits to control serum lipid levels in certain diseases and inhibit growth of colon cancer cells. Therefore, KO may be a good candidate for development as a functional food and nutraceutical".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Fukunaga 2008 [5[	Sixty weanling male F334 rats	4w	1) DHA-EE (0.051% of diet) 2) EPA-EE (0.051%) 3) Squid meal PC (0.1375%) (rich in DHA) 4) Starfish PC (0.1375%) (rich in EPA) 5) Corn oil 7%, (also added to all groups).	The purpose of this study was to investigate growth inhibition and apoptosis inducing effects of n-3 PUFA bound to PC from marine sources on chemically induced colon cancer in rats.	Squid and starfish PC inhibited the growth of Caco-2 cells. The diets suppressed colon cancer in rats. Rats consuming n-3 diets showed increased apoptosis and suppressed proliferation.		"These results suggest that marine PC-containing diets might be an effective dietary protective factor against colon cancer".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Tandy 2009 [6]	30-50 6w old male C57BL/6 mice	8 w	1.25%, 2.5% and 5% krill oil	To investigate the effects of dietary krill oil on cardiometabolic risk factors in mice fed a high-fat diet	KO supplementation reduces hepatic steatosis, glycemia, and hypercholesterolemia in high-fat-diet-fed mice.	"These data raise the possibility that n-3:PL or n-3:PC (found in KO) may be more efficacious than n-3:TG (found in FO)-a supposition that needs to be verified in future studies"	"These results demonstrate that dietary KO is effective in improving metabolic parameters in mice fed a high-fat diet, suggesting that KO may be of therapeutic value in patients with the metabolic syndrome and/or nonalcoholic fatty liver disease".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Batteta 2009 [7]	18 male Zucker rats 4 w old	4 w	Fish oil and krill oil both had 0.5 g / 100 g diet of EPA+DHA (0.8% of energy)	To compare the effect of fish oil vs. krill oil on ectopic fat and inflammation in rats.	"Our data suggest that the beneficial effects of a diet enriched with n-3 are the result of changes in membrane FA composition. The reduction of substrates for inflammatory molecules and endocannabinoids (ECs) may account for the dampened inflammatory response and the physiological re-equilibration of body fat deposition in obese rats".		"In conclusion, we have reported that diets rich in n-3 LCPUFA, and a KO-based diet in particular, exert beneficial effects on several metabolic dysfunctions in Zucker rats"

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Hossain 2009 [8]	60 male BALB/c mice 6w old	35 days	Squid PL liposomes 1.0 mg/mL, chitosan alone 5.0 mg/mL, squid PL liposomes 1.0 mg/mL with chitosan 5.0 mg/mL in the form of a simple mixture or squid PL liposomes 1.0 mg/mL coated with chitosan 5.0 mg/mL.	To assess the antitumor effects of chitosan-coated liposomes in an animal model of myeloma.	Inhibition of tumor growth was found to be through reduction in metal metalloprotein ase (MMP2 and MMP9) activity.		"Chitosan-coated marine phospholipid liposomes might be useful as potential agents for the treatment of myeloma SP2"
Di Marzo 2010 [9]	18 male Zucker rats 4 w old	4 w	Fish oil and krill oil, both at 0.5 g / 100 g diet of EPA+DHA (0.8% of energy)	To measure levels of n-3 and EC profiles in rat brains following fish- or krill-oil intake	"We conclude that... in the brain only 2-AG is affected by dietary krill oil, suggesting that the beneficial effect of the latter on the metabolic syndrome is mostly exerted by modifying peripheral ECs".	"Possible effects of dietary krill oil in the brain through modification of 2-AG levels deserve further investigation".	"In conclusion, we have reported here that one month administration to Zucker rats of a relatively low dose of KO... in the brain reduces only 2-AG levels, without significantly affecting AEA levels and food intake".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Irena 2010 [10]	10 2M old male Wistar rats and 42 3.5w old male DBA/1 mice	~60 d	Rats: 2.5% krill oil Mice: fish oil (0.47 g/100g) or krill oil (0.44 g/100g) EPA+DHA (0.8% of energy)	Effect of fish- or krill-oil on arthritic symptoms in a model of Rheumatoid Arthritis	Mice fed KO demonstrated lower infiltration of inflammatory cells into the joint and synovial layer hyperplasia.	"The presence of EPA, DHA and arachidonic acid in neutrophil phospholipids after KO and FO administration should be investigated in future studies".	"The study suggests that krill oil may be a useful intervention strategy against the clinical and histopathological signs of inflammatory arthritis"
Burri 2011 [11]	30 male CBA/J mice 2 M old	3 m	Fish oil (1.1%) or krill oil (1.5%). Alternatively: 0.31 g/100 g diet of EPA+DHA (fish oil) or 0.29 g/100g diet of EPA+DHA (krill oil)	Effect of fish- or krill- oil on glucose and lipid homeostasis and modulation of inflammation	Key metabolic pathways regulated by KO include glucose metabolism, lipid metabolism and the mitochondrial respiratory chain.	"Further studies of KO using animal models of metabolic disorders and/or diets with a higher level of fat may be required to observe these effects".	"Our data demonstrate a marked effect of KO on the regulation of genes and pathways involved in hepatic energy metabolism".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Fosshaug 2011 [12]	173 male Wistar rats	8w	0.47 g/100g diet of EPA+DHA (0.75% of energy) in the form of krill oil	Effects of krill oil on cardiac remodeling after experimental myocardial infarction (MI).	Treatment with krill oil before MI leads to attenuated left ventricular (LV) dilatation and hypertrophy in rats.	"Future studies are needed to establish whether these beneficial effects are consequences of attenuated cardiac remodeling or reduction of MI sizes. Also, the molecular effects of krill oil on the heart are not yet clear and need to be examined further".	"Supplement with krill oil leads to a proportional increase of n-3 PUFA in myocardial tissue and supplement given before induction of MI attenuates LV tissue remodeling"

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Gamoh 2011 [13]	42 male Wistar rats	3 w	Krill oil at doses of 300 mg EPA + 120 mg DHA 215 mg EPA + 86 mg DHA	Effects of krill oil on spatial learning ability			"Chronic administration of krill oil improves spatial-memory related learning ability in the similar way as ethyl ester form of EPA or DHA...krill is a good source of high-quality protein and n-3 PUFAs; therefore, it may become an important source of nutrition in the future".
Lukas 2011 [14]	Growing (age 28 d) female Sprague–Dawley rats (n=60)	8 w	High fat diet containing corn oil, flaxseed oil, menhaden oil (7.2% EPA+DHA), krill oil (17.8% EPA+DHA), salmon oil (11.9% EPA+DHA) or tuna oil (5.7% EPA+DHA)	To determine the effect of various n-3 PUFAs sources on bone during growth.	"In our study, greater tibia length in growing rats fed TO may be due to DHA promoting bone growth by activating osteoblasts in the periosteum"	"Further studies are needed to address this issue".	"The animal study results suggest consuming a variety of n-3 PUFA sources to promote bone health during the growth stage"

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Piscitelli 2011 [15]	30-50 male C57BL/6 mice 6 w old	8 w	1.25%, 2.5% and 5% krill oil	Dose-dependent effects of krill oil on metabolic parameters in high fat diet fed mice	"Our data suggest that KO may promote therapeutic benefit by reducing EC precursor availability and hence EC biosynthesis"		"In conclusion, our data have shown that...n-6 PUFAs dietary content influences EC precursors and biosynthesis, and that the addition of KO to the diet can ameliorate several metabolic disturbances and reduce EC levels in most of those peripheral organs the malfunctioning of which is responsible for such disturbances".
Tou 2011 [16]	60 female Sprague-Dawley rats age 28 d	8 w	Corn oil and flaxseed oil (both 12%), krill oil (10% + 2% corn oil), menhaden oil (10% + 2% corn oil), salmon oil (SO) (12%) or tuna oil (TO) (12%)	To determine the effect of different sources of n-3 PUFAs on digestibility, tissue deposition, eicosanoid metabolism, and oxidative stability.		"Further studies are needed to clarify whether different PLs influence fatty acid digestibility".	"On the basis that the optimal n-3 PUFA sources should provide high digestibility and efficient tissue incorporation with the least tissue lipid peroxidation, TO and SO appeared to be the most beneficial of the n-3 PUFAs sources evaluated in this study".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Ferramosca 2011 [17]	12 male Wistar rats	Up to 6 w	Fish oil or krill oil (~0.5% EPA+DHA), or olive oil (placebo)	Effect of fish oil and krill oil on modulation of hepatic lipogenesis	In rats fed with KO, a time-dependent decrease in the activities of the mitochondrial tricarboxylate carrier and of the lipogenic enzymes was found, caused by a reduced expression of the protein.		"We believe that the present investigation opens up new possibilities regarding the use of dietary KO as a preventive factor for dyslipidaemia".
Ferramosca 2012 [18]	130 Male Sprague-Dawley rats	12 w	Standard diet (6% fat), high fat diet (35% fat) and HF diet + krill oil (2.5% krill oil, 0.5% EPA+DHA)	Effect of krill oil on hepatic steatosis	Investigation of the molecular mechanisms of KO action revealed a strong decrease in the activities of the mitochondrial citrate carrier and of the cytosolic acetyl-CoA carboxylase and fatty acid synthetase, which are both involved in hepatic de novo lipogenesis	"In view of the results reported in this pre-clinical study, further clinical studies are warranted to confirm the effects of KO on human metabolism".	"It became evident that KO positively influences many metabolic steps in a way that counteracts the potentially negative effects of a hypercaloric and hyperlipidic diet, which often characterizes the nutritional habits of western populations".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Grimstad 2012 [19]	30 male Wistar rats 12 weeks old	29 d	Control, control + DSS (inducer of colitis) @ d23, 5% krill oil + DSS @d23	To evaluate the effects of krill oil on inflammation and redox status in a model of colitis in rats	"KO showed protective potential against DSS colitis based on the preservation of colon length, reduction of oxidative markers and the consistent beneficial changes of HCS, cytokine, and PGE3 levels, as well as PPAR-γ and Pparγ1α expression compared with DSS alone".	"As KO may attenuate inflammation and decrease protein oxidative stress in experimental colitis, larger studies are of interest in both IBD animal models and in humans with IBD"	"These findings indicate an anti-inflammatory and a protein antioxidant effect of KO"

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Bjørndal 2012 [20]	16-20 transgenic male C57BL/6 mice constitutively expressing hTNFα	6w	high-fat diets (23.6%,w/w) with or without krill **powder** (6.4% lipids, 4.3% protein, w/w)	To investigate hepatic regulation of energy metabolism after feeding a powder isolated from Antarctic krill	Krill powder caused reduction in plasma TG and cholesterol, possibly due to down-regulation of hepatic expression of genes involved in lipogenesis and glycerolipid synthesis, and increased β-oxidation activity. Genes involved in glycolysis and gluconeogenes is were significantly reduced in liver by the krill powder diet.	"To further explore the effects of a high-energy intake on metabolism, studies in models with obesity related inflammation should be conducted".	"In a high-fat mouse model with disturbed lipid metabolism due to persistent hTNFα expression, krill powder showed significant effects on hepatic glucose- and lipid metabolism, resulting in an improved lipid status in liver and plasma".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Rossmeisl 2012 [21]	12-21 male C57BL/6J mice	1st study 9w, 2nd study 4m	Fish triglyceride or fish phospholipids (EPA+DHA 10 and 30 g/kg diet)	To study the effects of omega-3 PLs compared with omega-3 TGs on obesity-associated disorders.	"Multiple mechanisms may be responsible for the relatively strong biological effects of omega-3 PL of marine origin, including: (i) the effect of this molecular form on absorption, transport and organ distribution of n-3 FA, namely the superior bioavailability of DHA and especially EPA; (ii) relatively strong depression of EC system activity in the tissues by n-3 PL; and (iii) regulation of cellular metabolism by yet unidentified n-3 PL species functioning as ligands to specific nuclear receptors".	"Future studies regarding hepatic effects of omega-3 PL might reveal novel targets for treatment of insulin resistance"	"Compared with triglycerides, dietary DHA/EPA administered as phospholipids are superior in preserving a healthy metabolic profile under obesogenic conditions, possibly reflecting better bioavalability and improved modulation of the EC system activity in white adipose tissue".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Li 2013 [22]	48 male Wistar rats 4 w old	4 w	PKO – ethanol extraction of oil from krill mil, WKO – hexane extraction from krill mil. PKO had 30-40% more n-3 than WKO. All rats were fed high cholesterol diet followed by krill oil diet: 50, 200 and 400 mg/kg WKO and 50, 200 and 400 mg/kg PKO	To investigate the effects of KO intake on plasma cholesterol and glucose levels in rats fed a high-cholesterol diet		"The exact mechanism behind the bioactivity of KO deserves further study".	"PKO showed better overall cholesterol-lowering effects than WKO, which may be due to its higher n-3 PUFA levels".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Vigerust 2013 [23]	26 6-8w old male transgenic mice expressing human TNF-a	6 w	Fish oil (EPAX 2.9% w/w). Krill oil (5.8 % w/w).	To investigate the effect of fish oil and krill oil on lipid homeostasis and inflammation	KO was capable of modulating lipid metabolism by lowering plasma levels of TAG and cholesterol and stimulating the mitochondrial and peroxisomal fatty acid β-oxidation, as well as improving the overall carnitine turnover.	"The effect of dietary oils on the levels of inflammatory markers in hTNF-α transgenic mice fed high-fat diet needs further investigations".	"Our findings demonstrate that FO and KO are comparable dietary sources of n-3 PUFAs. However, when quantitatively similar doses of n-3 PUFAs are administered, KO seems to have a greater potential to promote lipid catabolism..."

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Wibrand 2013 [24]	38 male and 38 female adult Wistar-Unilever rats aged 6 w	7 w	Krill oil (~0.2g krill oil /day /animal) or 1.25% of daily ration	To evaluate the effects of krill oil on cognition and depression-like behavior in rats	Imipramine and KO treatments are associated with enhanced *Bdnf* mRNA expression but have distinct effects on the expression of *Arc* and other synaptic-plasticity associated genes, suggesting partially distinct neurobiological mechanisms.		"These results indicate that active components (EPA, DHA and astaxanthin) in KO facilitate learning processes and provide antidepressant-like effects".
Bjørndal 2013 [25]	10-12 male CBA/J mice	3m	Low-fat control diet or a 3% (w/w, 1.9% lipid and 1.1% protein) low-fat krill powder diet	To study the effect of krill powder on hepatic gene expression in mice	"Krill powder supplemented diet had potent and specific effects on energy metabolism and oxidative phosphorylation at the gene level".	"In further studies it will be interesting to investigate the effect of krill oil and krill powder on the development of insulin resistance and type-2 diabetes mellitus".	"Krill powder supplementation could be an approach to prevent decline in mitochondrial respiratory chain function"

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Hu 2013 [26]	40 Male Sprague-Dawley rats	2m	Low dose (25 mg/kg body weight) or high dose (75 mg/kg body weight) PC from sea cucumber (*Cucumaria frondosa*)	Effect of PC-EPA from sea cucumber in an animal model of hyperglycemia	"Cucumaria-PC exhibited significant anti-hyperglycemic activities through up-regulating PI3K/PKB signal pathway mediated by insulin".	"Further in-depth investigations are needed to better understand the effect of Cucumaria-PC on GLUT4 translocation to the plasma membrane "	"Nutritional supplementation with Cucumaria-PC, if validated for human studies, may offer an adjunctive therapy for diabetes mellitus".
Rossmeisl 2013 [27]	37 male C57BL/6N mice	7w	*PL-DHA/EPA from Herring (5 g/kg diet omega-3) *Diet with rosiglitazone (10 mg/kg diet) *Diet with rosiglitazone (10 mg/kg diet) + PL-DHA/EPA from Herring (5 g/kg diet omega-3)	Characterize the mechanisms underlying beneficial effects of DHA/EPA PLs, alone or combined with an antidiabetic drug (rosiglitazone), on hepatosteatosis.	"The complex down regulation of hepatic lipogenic and cholesterol biosynthesis genes and the antisteatotic effects were unique to DHA/EPA-containing phospholipids, since they were absent in mice fed soy-derived PC".	"The liver weight was similar in the HFD-fed and control mice despite a marked hepatic steatosis in the former mice. The origin of this phenomena remains to be further explored".	"Inhibition of lipid and cholesterol biosynthesis associated with potent antisteatotic effects in the liver in response to DHA/EPA-containing phospholipids support their use in non-alcoholic fatty liver disease prevention and treatment".

Summary of Pre-Clinical Studies Evaluating the Effect of Marine Phospholipid oil on health related issues							
Study	**Population**	**Duration**	**Dose**	**Study Objective**	**Mechanism**	**Future studies**	**Study Conclusions**
Liu 2014 [28]	28 male C57BL/6J mice	8w	High fat diet containing 2 % DHA-PL (from squid, *Sthenoteuthis oualaniensis*) or High fat diet containing 2 % EPA-PL (from sea cucumber, *Cucumaria frondosa*)	To compare the effects of PL-DHA and PL-EPA from marine sources on obesity-related metabolic disorders	DHA-PL and EPA-PL up-regulated genes involved in insulin-sensitizing actions in the adipose tissue and suppressed hepatic SREBP-1c mediated lipogenesis. EPA-PL also significantly activated PPARα mediated fatty acid β-oxidation in the liver.	"Further studies are required to clarify the diverse effects of dietary DHA-PL and EPA-PL on the uptake, synthesis and excretion of cholesterol in the liver".	"These results indicate that DHA-PL and EPA-PL could efficaciously alleviate obesity-related metabolic disorders but the ameliorative degree and regulatory mechanisms are not identical".
Wu 2014 [29]	20 4M old SAMP8	12w	Control and PL-EPA (from the sea cucumber *Cucumaria frondosa*) (0.5% of diet)	To investigate the effect of EPA-enriched PLs from sea cucumber on learning and memory functions in mice	"The neuroprotective activity of EPA-enriched PL might be mediated, in part, via inhibition of the mitochondria-dependent apoptotic pathway".	"Future studies are necessary to thoroughly understand the mechanisms involved in the effects of EPA-enriched PL in PC12 cells and SAMP8 mice".	"Our results indicated that EPA-enriched PL could offer an efficient and novel strategy to explore novel drugs or functional food for neuronprotection and cognitive improvement".

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Appendix III. Compositional comparison between OmegaPC™ and krill oil

	OmegaPC	Lecithin derived from krill (GRN 226 – grade A)	Superba™ (GRN 371)	NKO™ (GRN 242) *
Phospholipids (%w/w)	39	40-50	44.7	45.3
Glycerides (tri-, di-, and mono-) (%w/w)	47	43.9	38.8	40.6**
Total omega-3 (%w/w)	25	19.7	23.5 ±2	33.1
DHA (%w/w)	12	5.3	6.5 ±1	11
EPA (%w/w)	10	10.3	14 ±2	17.3

*Numbers represent average values based on data included in the GRAS notice.

**Calculated based on average total lipids minus average PL

SUBMISSION END